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Contents

Preface

Plant physiology is a complex combination of biology, chemistry, and physics which describes why plants grow the way they do. Biological aspects include basic cellular functions and processes of life. Chemical aspects include the behavior of substances based on their chemical properties and their importance in plant growth and development. Physics plays a key role in helping to explain the behavior of light and water with respect to plants. Understanding what happens in a plant in response to horticultural practices helps us to understand why we do what we do. With an understanding of 'why' we can make better decisions with respect to 'what', 'how', and 'when'.

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1 Horticulture – Whole Plant Integration of Many Disciplines

Horticulture is a unique combination of art and science. Students from many disciplines blend math, science and art skills to enrich our world. The design and aesthetics of horticulture rely on artistic abilities while understanding plant function requires a strong science and math background.

A basic understanding of plant anatomy and physiology are acquired by studying botany and its allied disciplines. In order to understand the complex nature of plant physiology, strong foundations in chemistry and biochemistry are needed. Environmental influences of light and temperature on plant growth, development and productivity are readily comprehended with an appreciation of the physics involved.

Most students specialize in one of the five major branches of horticulture: (i) pomology; (ii) olericulture; (iii) floriculture; (iv) ornamental, nursery and landscape plants; and (v) landscape architecture (Fig. 1.1).

Pomology is the study of fruit production and is usually subdivided into tree and small or bush fruits. The major tree fruit crops normally include almond (*Prunus dulcis*), apples (*Malus domestica*), apricots (*Prunus armeniaca*), banana and plantains (*Musa* spp.), cacao (*Theobroma cacao*), cashew (*Anacardium occidentale*), cherries (*Prunus avium, Prunus cerasus*), citrus (*Citrus* spp.), coconut (*Cocos nucifera*), coffee (*Coffea arabica, Coffea canephora*), date (*Phoenix dactylifera*), hazelnut (*Corylus avellana*), macadamia (*Macadamia integrifolia, Macadamia tetraphylla*), mango (*Mangifera indica*), oil palm (*Elaeis guineensis*), olive (*Olea europaea*), papaya (*Carica papaya*), peaches and nectarines (*Prunus persica*), pear (*Pyrus communis, Pyrus pyrifolia*), pecan (*Carya illinoensis*), pistachio (*Pistacia vera*), plums (*Prunus domestica, Prunus salicina*) and walnut (*Juglans* spp.). The small, or bush fruit, include blackberries (*Rubus* spp.), blueberries (*Vaccinium* spp.), cranberries (*Vaccinium macrocarpon*), currants (*Ribes* spp.), gooseberries (*Ribes* spp.), grapes (*Vitis* spp.), kiwifruit (*Actinidia* spp.), raspberries (*Rubus* spp.) and strawberries (*Fragaria* × *ananassa*). Fruit crops may also divided into region of adaptation, namely temperate, subtropical and tropical. Most fruit crops are perennial or biennial although strawberries may be grown as an annual in many regions of the world. Most fruit is field grown, with the notable exception of greenhouse-produced strawberries and raspberries. Small fruit culture is ideally suited for protected culture using high tunnels.

Olericulture is the study of vegetable production. Most vegetable crops are annuals, although there are some perennials, such as asparagus (*Asparagus officinalis*). Some vegetables such as beets (*Beta vulgaris*), carrots (*Daucus carota*) and cabbage (*Brassica oleracea*) are botanically biennial, but are grown and harvested as annuals. Most vegetable production is field oriented. Tomatoes (*Solanum lycopersicum*) and peppers (*Capsicum annuum*) are notable exceptions to this, and many crops are forced early or grown under a lengthened season through the use of unheated tunnels.

Floriculture is the study of flower production, either in the field or in greenhouses.

The study of ornamental, nursery and landscape plants makes up the fourth branch of horticulture. This branch is often intimately linked to landscape architecture since the plants involved in this branch are the materials used in the landscape.

The study of landscape design and implementation is collectively known as landscape architecture. Landscape architects not only have to know how to integrate plant materials into our surroundings, but also, which ones are most suitable for specific sites.

Plant Anatomy Review

A plant's function is intimately linked to its structure. In order to understand how an organism works, we

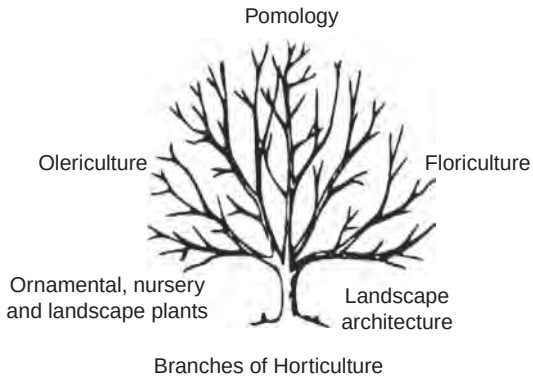

Fig. 1.1. The major branches of horticulture.

dissect it and study its individual pieces. How do we know what we know? We study the structures in plants using either light or electron microscopes and we study the functions of the various parts using experiments involving cell parts, whole cells, tissues or whole plants. Students are referred to *Esau's Plant Anatomy* by Evert and Eichorn (2006), who re-wrote Katherine Esau's classic text, if they are in need of more than a review or would like to see illustrations of many of the cellular components.

Plant Parts

Cells and their parts

The basic building block of all living organisms is the cell. Each individual structure in the cell plays a unique role in the overall function of the cell (Table 1.1). The integration of these individual parts into a living cell is one of the wonders of biology. There are many cell types, shapes and sizes. To gain a perspective on just how small an individual cell is, consider that 150,000,000 typical plant cells would fit into a space approximately 3 cm^3.

The cell wall

If we looked at one individual plant cell and worked our way inward, we would first observe the outer box-like structure called the cell wall. This structure is unique to plants; animals do not have cell walls. This 'box' is made up of cellulose fibers (long molecules of glucose attached to each other in a way that humans cannot digest) glued together with carbohydrate-based substances known as lignins and pectins. The cellulose in the cell wall is what we often refer to as roughage or fiber. (Cellulose is insoluble fiber, important in maintaining intestinal regularity. The other type of fiber, soluble fiber, has important health benefits that will be discussed in Chapter 17, this volume.)

The cell wall is important for giving plants their rigidity and structure. By being rather rigid, cell walls allow pressure to build up within the cell itself. This pressure, called turgor pressure, is responsible for the upright nature of most plants. When water

Table 1.1. The major parts and functions of a typical plant cell.

Cell component	Function(s)
Cell wall	Contains the rest of the cell Allows build up of turgor pressure
Plasmalemma	Encloses the living entities of the cell Selectively permeable to regulate influx and efflux from protoplasm
Plasmodesmata	Connects adjacent cells to allow intercellular communication
Nucleus	Stores the genetic material for cell structure and function in the form of DNA
Ribosome	Responsible for protein synthesis
Endoplasmic reticulum	Location of many ribosomes Important in membrane and lipid synthesis
Vacuole	Contains many products of cellular metabolism Important in regulating cellular turgidity
Plastids	Synthesis and/or storage of cellular products Photosynthesis
Leucoplast	Monoterpene synthesis
Chromoplast	Store pigments
Elaioplasts	Store lipids
Proteinoplast/ aleuronoplasts	Store protein
Amyloplast	Store starch Important in geotropism
Chloroplast	Photosynthesis
Golgi bodies	Production of cell wall materials
Mitochondria	Respiration

is lacking, turgor pressure often decreases, which reduces the plants' rigidity causing them to wilt.

The cell wall is the non-living part of the cell. It is the container in which the living parts of the cell, the plasmalemma and all components contained therein, are enclosed.

The plasmalemma

The plasmalemma is a double-layered membrane containing all the cell parts interior to the cell wall. Think of it as a 'bag' of water and dissolved substances in which all the other cell parts reside. Each cell part has a unique function, and when they are all integrated, that creates the living cell. The plasmalemma selectively regulates what moves into and out of a cell.

In addition to its regulatory function, the plasmalemma allows pressure to build up due to turgor. Think of the plasmalemma as a balloon. Only a certain amount of pressure could build up in the 'balloon' before it would burst if there was not a 'box' containing it. Thus it is the combination of pressure build up inside the plasmalemma and the resistance to that pressure afforded by the cell wall which gives plants their rigid nature.

The plasmalemma is selectively permeable due to its structure. It is essentially two sheets of lipids floating against each other. These lipids are hydrophobic, thus they keep water and anything dissolved in water either inside or outside the cell. Embedded across these two sheets of lipids, are proteins. Proteins are hydrophyllic, thus they allow water and solutes to move through them and into or out of the cell. Of course the solute molecules have to be small enough to fit through the spaces within the protein structure to move in either direction. Movement of molecules may also be regulated by concentration and electrical charge gradients across the membrane.

The plasmalemma together with everything inside it is called the protoplast. Thus your typical plant cell consists of a protoplast surrounded by a cell wall.

The plasmodesmata

There are numerous outgrowths of the plasmalemma which connect adjacent cells to each other. These membrane outgrowths are called plasmodesmata. The plasmodesmata allow for communication between cells via movement of substances from one cell to another. This movement may be due to normal cellular processes or it might be triggered by some environmental stimulus.

The protoplasm

We call the watery liquid inside the plasmalemma which contains the other cell parts the protoplasm. It is a mixture of water and dissolved substances including sugars, proteins, and salts. If we wish to refer to the watery liquid inside the plasmalemma but outside the nucleus, we would call it the cytoplasm. The liquid inside the nucleus is the nucleoplasm. Thus the nucleoplasm plus the cytoplasm is collectively the protoplasm.

Organelles

Organelles are individual membrane-bound components of the cell. Each organelle has its own membrane. Some organelles synthesize while others catabolize substances. Thus the membranes help to compartmentalize the cell preventing metabolic chaos.

THE NUCLEUS The nucleus is a membrane-bound organelle in which the genetic material for cell structure and function is stored in the form of deoxyribonucleic acid or DNA. This DNA stores the code for proteins, of which many are enzymes, and these enzymes run the cellular show. Normally, the DNA is not visible under a microscope. During cell division, the DNA becomes visible as chromosomes.

The membrane surrounding the nucleus is called the nuclear membrane. During normal cellular activity, the message coded in DNA is translated into ribonucleic acid or RNA. The RNA codes for specific sequences of amino acids which ultimately give rise to proteins and then enzymes outside of the nucleus at the ribosome. The particular sequence of amino acids imparts structure to the enzymes and the structure ultimately imparts a function.

RIBOSOMES AND THE ENDOPLASMIC RETICULUM Ribosomes are responsible for protein synthesis. Ribosomes may be free-floating in the cytoplasm or may be attached to a long string-like membrane called the endoplasmic reticulum, which is also found in the cytoplasm.

THE VACUOLE The organelle which often occupies the greatest amount of cellular space is the

vacuole. The vacuole is a large, membrane-bound organelle full of many products of cellular metabolism. The membrane of the vacuole is called the tonoplast. Since it contains many water-soluble components, the vacuole is important in cellular turgidity and water relations of plants. In fact, it is the vacuole which imparts the greatest influence on turgidity in the cell.

PLASTIDS Plastids are small, membrane-bound organelles whose primary function is storage of specific cellular products. Their name reflects their content. Leucoplasts are colorless plastids that primarily synthesize monoterpenes. Elaioplasts store lipids and proteinoplasts (aleuronoplasts) store proteins. Chromoplasts are plastids containing pigments and are often found in flower petals and fruit. Amyloplasts are plastids containing starch and are often found in root cells or immature starchy fruit. Amyloplasts are responsible for geotropic responses.

Chloroplasts are green plastids containing the pigment chlorophyll. Chloroplasts are the site of photosynthesis and are particularly abundant in leaves. Two important types of chlorophyll, 'a' and 'b' trap light energy and that energy is ultimately stored (often as a carbohydrate, fat or protein) in the plant or used for metabolism. Chloroplasts contain their own DNA and reproduce on their own. There may be from one to more than 100 chloroplasts in a cell.

There are two membranes surrounding the chloroplast, an inner and an outer chloroplast envelope. Another membrane inside the chloroplast has flat vesicles called thylakoids. These thylakoids are stacked in pancake-like structures called grana. The grana provide a large surface area for maximum light interception. The membrane that forms the thylakoid is called the lamella and the liquid in which grana reside is called the stroma.

GOLGI BODIES Golgi bodies are organelles that are responsible for the production of materials used in cell wall construction.

MITOCHONDRIA The mitochondria are the organelles responsible for the controlled release of energy (respiration) from food within the cell. The mitochondrion is a rod-shaped organelle with a single outer membrane and a highly folded inner membrane. These folds on the inner membrane are called cristae and the liquid inside the mitochondria is called the matrix. There are many mitochondria in a cell.

Cell types

As previously noted, there are many different types of cells in a plant (Table 1.2). They each have a different structure which conveys to them different functions.

Parenchyma

Parenchyma cells are usually relatively large, isodiametrically shaped cells with thin cell walls. They often function as storage cells.

Collenchyma

Collenchyma cells are thick-walled cells providing support to tissues, especially young, actively growing leaves and stems. The cell walls of collenchyma remain pliable, thus allowing for cellular expansion during growth. Both parenchyma and collenchyma cells can resume meristematic activity if needed, which is important for wound healing and cork formation.

Sclerenchyma

Sclerenchyma are also thick-walled cells that provide support, but their walls are not pliable and they often lack a protoplast at maturity, thus they cannot resume meristematic activity. At maturity they are considered non-living. Two distinct types of sclerenchyma are sclereids and fibers. Both sclereids and fibers offer support to plant tissues. Sclereids are often isodiametric in shape, but may also be somewhat elongated. Fibers tend to be many times longer than they are wide. While sclereids provide support in many different plant tissues, fibers are particularly important in support of vascular tissues.

Fibers are often associated with the bast fibers of commerce. Phloem fibers from dicots are often referred to as soft fibers since they remain soft and pliable at maturity. Fibers from monocots are often called hard fibers since they are stiff and non-pliable. Sometimes the fibers of commerce are not really even fibers at all. For example, cotton is composed of the epidermal hairs on seeds.

Table 1.2. Plant cell types and their function.

Cell type	Function(s)
Parenchyma	Storage of cellular metabolites Capable of meristematic activity
Collenchyma	Provide support Capable of meristematic activity
Sclerenchyma (sclereids and fibers)	Provide support
Tracheids	Lateral water and solute transport in xylem
Vessel elements	Lateral and longitudinal water and solute transport in xylem
Sieve elements	Transport of photosynthetic products
Companion cells	Provide energy to sieve elements for transport

Cells of the xylem

TRACHEIDS Tracheids are specialized cells of the xylem whose function is to transport water and solutes throughout the plant. They are characterized as elongated, thick-walled cells with pits. Pits are areas on the lateral external edges of some cell walls where secondary cell-wall thickening did not occur. Pits often occur in the same location of two adjacent cells and are called pit pairs. Pit pairs allow for the movement of substances transversely from cell to cell in the xylem. Functioning tracheids are non-living as the functioning protoplasm disintegrates upon cell maturity.

VESSEL ELEMENTS Tracheids that have perforations in the end walls of their cells are called vessel elements. Two or more vessel elements connected to each other are called vessels. Water movement in the xylem through tracheids occurs via the pit pairs. In vessels, water movement may be via pit pairs or longitudinally through the perforations in the end walls.

Cells of the phloem

SIEVE ELEMENTS Sieve elements are highly specialized cells in the phloem existing as sieve cells and sieve-tube members. Their function is to actively transport the products of photosynthesis to sites of utilization and storage within the plant. Both sieve cells and sieve-tube members are characterized as thick-walled, elongated cells with modified protoplasts. The protoplasts of laterally or vertically joined sieve elements are connected through pores in the cell walls. The areas on a cell wall where pores occur are called sieve areas. These pores are often much larger than the pores required for intercellular connections via plasmodesmata, and thus the two types of intercellular connections are different. Since functioning protoplasts are present at maturity, sieve elements are living, unlike the functioning cells of the xylem.

When the sieve areas of sieve elements are not very well developed or congregated at any specific area on the cell wall, the cells are called sieve cells rather than sieve elements. When the sieve areas are highly developed and tend to occur mostly on end walls, the cells are called sieve-tube members. A number of sieve-tube members vertically connected is called a sieve tube. Generally speaking, most gymnosperms have sieve cells while angiosperms have sieve tubes.

Protoplasts of both types of sieve elements change rather dramatically during maturation. Their nucleus, endoplasmic reticulum and ribosomes disappear. Additionally, the vacuole tonoplast disintegrates, leaving the plasmalemma to regulate cell turgidity. Starch accumulating plastids are retained (amyloplasts) and mitochondria are present. Thus the sieve cells or sieve-tube members have a plasmalemma with cytoplasm containing plastids and mitochondria.

COMPANION CELLS Specialized parenchyma cells associated laterally with sieve elements are called companion cells. Their function is to provide the cellular energy that is required for the movement of substances in the phloem. In gymnosperms, companion cells are called albuminous cells. Since movement of substances in the xylem does not require metabolic energy, there are no companion cells associated with functioning xylem cells.

Tissues

Tissues are highly organized groups of cells that perform a specific function. Each cell within the tissue has the basic cell attributes previously described yet each may have a specific function. All cells of the tissue are integrated in such a way that the tissue's function is achieved (Table 1.3).

Table 1.3. Plant tissues and their function(s) with general cellular composition of each tissue.

(a) Simple tissues (one cell type)

Plant tissue	Function(s)	Cell type(s)
Parenchyma	Storage Cell division	Parenchyma
Sclerenchyma	Support	Sclerenchyma
Collenchyma	Support	Collenchyma

(b) Complex tissue (more than one cell type)

Plant tissue	Function(s)	Cell type(s)
Epidermis	Protection from the elements Gas exchange Absorption of water and minerals Production of secretory metabolites	Parenchyma, guard cells, trichomes, root hairs
Vascular	Transport of water, nutrients, photosynthates, chemical messengers	Parenchyma, vessel elements, tracheids, sclereids, fibers, sieve elements, sieve-tube members, companion cells, fibers
Meristematic	Cell division	Initials, derivatives
Bark	Outer protective layer of woody plants	Phellogen, phelloderm, phellum, phelloid

Simple tissues – one cell type

Tissues in which there is only one cell type are called simple tissues. There are three simple tissues in plants: (i) parenchyma; (ii) sclerenchyma; and (iii) collenchyma. Notice how the tissue name indicates the cell type of the tissue.

PARENCHYMA The function of parenchyma is storage. The main substances stored in parenchyma are starch, fats, and proteins, depending on the organ the tissue is located in and the stage of plant development.

SCLERENCHYMA Sclerenchyma provides rigid strength to plant organs, especially in organs where further cell expansion is not likely to occur. Sclerenchyma is also quite evident in certain fruit. The pits of stone fruit such as peaches or cherries are composed primarily of sclerenchyma. In this case, sclerenchyma is providing protection to the developing seed inside the pit. Pear and quince (*Cydonia oblonga*) fruit have gritty textured sclerenchyma cells throughout the otherwise parenchymatic flesh. Pear, quince and apple cores also have significant sclerenchyma in them. Seed coats of many species have considerable sclerenchyma in them, providing protection to the enclosed embryo.

COLLENCHYMA Collenchyma provides flexible strength to plant organs, especially leaves and stems when expansion growth is likely to occur. The stalk (leaf petiole) of celery (*Apium graveolens*) has numerous areas of collenchyma tissue in it providing support and crunch.

Complex tissues – more than one cell type

Complex tissues generally have more than one cell type.

EPIDERMIS The epidermis is the outer protective layer on both roots (woody and herbaceous) and shoots (herbaceous) characterized by a layer of a fatty substance called cutin. Cutin helps reduce water loss through the epidermis and also helps prevent the intrusion of pathogens.

The epidermis is usually only one cell layer thick. Some plants have a multilayer epidermis, but this is relatively rare. An example is *Ficus elastica*. Epidermal cells are usually rectangular parenchyma cells, however, other cell types are also found in the epidermis. In general, epidermal cells do not photosynthesize to any great extent. An exception is that epidermal cells of many shade plants exhibit significant photosynthesis.

While the principle function of the epidermis is to create a barrier to the external environment, there are several other specialized functions of the

epidermis due to the existence of highly specialized cells. These cells include root hairs, guard cells and trichomes.

Root hairs, as the name implies, occur in the epidermis of roots. They are irregularly shaped cells with a large surface area protruding into the soil matrix. Their function, due to the high surface area, is to increase absorption of water and solutes from the soil.

Guard cells form specialized pores called stomata in the epidermis. The function of stomata is gas exchange between the plant and the atmosphere. Thus, guard cells are most prevalent in the epidermis of leaves. They do occur on fruits and stems, but to a much lesser degree. Changes in the water content of guard cells determine whether or not the stomata are visible.

Many plants have specialized structures on the epidermis called trichomes. Trichomes can be single or multi-cellular. Root hairs are actually trichomes. Most trichomes secrete or store some secondary metabolite. Often, trichomes contain substances, which when released will deter feeding of insects. Thus at least one function of trichomes is in plant defense.

VASCULAR TISSUE Vascular tissue is responsible for long-range transport of substances in plants. Generally, xylem transports water and dissolved substances acropetally (from root to shoot tip) while phloem transports water and dissolved substances, primarily sugars, from the photosynthetic source (a leaf) to a sink (fruits, roots, developing tissue) in a basipetal direction. Movement in the xylem is passive and does not require metabolic energy while movement in the phloem is active and requires metabolic energy.

The main cell types in the xylem are vessel elements, tracheids, sclereids and fibers. Additionally, xylem contains parenchyma cells often protruding radially through the xylem. Their function is to store starch. The main cell types of the phloem include sieve elements, sieve-tube members, companion cells, fibers and sclereids.

MERISTEMATIC TISSUE Meristematic tissue is the tissue responsible for growth via cell division which occurs in many different parts of the plant. Meristematic cells are parenchyma cells which quickly differentiate into the many different cell types. Cells that retain the meristematic activity are called initials while those that begin differentiating are called derivatives. The vascular cambium is the tissue responsible for the production of xylem and phloem. Phellogen is tissue responsible for the production of cork and bark on woody stems. Apical meristems are responsible for growth at the apex of either roots or shoots. Increases in height or root depth are due to cell division in the apical meristem. (Of course cell division must be followed by cell elongation for significant root or shoot lengthening to occur.) Increases in root or stem circumference are due to the activity of vascular cambium and phellogen. The intercalary meristem or leaf cambium is responsible for cell division in leaf tissue. Buds are meristematic regions enclosed by protective bud scales. They are often categorized based on their ultimate fate (floral, vegetative or mixed) or their position (terminal, axillary or adventitious).

BARK Bark is the outermost protective tissue on woody plant stems. It is produced by the phellogen (cork cambium). Cells from the phellogen that are pushed inwards are called phelloderm. Other cells that are pushed outwards have a coating of suberin which is impermeable to water and gas. These cells soon die and become air filled and are called phellum cells (cork cells). Other cells produced by the phellogen and pushed outward that are not suberized are called phelloids. Since they lack suberin, they can exchange water, gas and nutrients, thus they are living.

Organs

Organs are major distinct and visibly differentiated groups of tissues characterized by a general purpose or function in the survival and/or reproduction of the plant. We generally consider six major organs of a plant: (i) roots; (ii) stems; (iii) leaves; (iv) flowers; (v) fruit; and (vi) seeds.

Roots

Roots are the main underground organ of the plant. Some roots are above ground, but most are beneath the soil surface. Roots anchor the plant in its growing medium. Roots are also responsible for the uptake and translocation of water and dissolved nutrients and may also serve as storage units for food reserves. Roots are also responsible for the production of growth regulating compounds, especially cytokinins.

Root growth is classified as either primary or secondary. Primary root growth is that root growth originating at the root apical meristem of the embryo in a seed. The root apical meristem is characterized by the presence of a protective root cap. This cap protects meristematic cells from the pressure generated by root elongation in the soil and also produces muscilage for lubricating the roots' penetration through the soil. The root cap is also responsible for geotropic responses via gravity-sensing plastids (amyloplasts). Primary root growth is also important in the production of growth regulators.

A cross-section of a primary root reveals three general regions: (i) the epidermis; (ii) the cortex; and (iii) the vascular region. The epidermis, as previously discussed, provides a barrier to the environment and also facilitates absorption from the soil via root hairs.

The cortex is a region of mostly parenchyma cells with the purpose of storage. The innermost layer of the cortex is called the endodermis. Cells of the endodermis have a covering of suberin called the casparian strip. This strip helps regulate water movement into the center of the root towards the vascular region. Just interior to the endodermis is the pericycle, the area of the cortex responsible for the origin of lateral roots.

The vascular region, located inside the pericycle is composed of xylem in the shape of a starfish, radiating out from the center of the root. The phloem is located between the arms of the xylem. The xylem and phloem together comprise the vascular cylinder which is called the stele.

Secondary root growth is growth which arises from vascular cambium and phellogen and results in an increase in root girth.

Adventitious root growth is root growth at sites on a plant not normally associated with root growth, such as at a node on a stem. Adventitious roots may remain primary or undergo secondary growth.

Roots have no leaves, nodes or internodes but may have buds. Roots with buds are often used in vegetative propagation.

Stems

Stems may be located above or below ground. Stems have leaves, buds, nodes and internodes and are thus easily distinguished from roots. Primary stem growth is from growth originating in the shoot apical meristem of an embryo and results in an increase in stem length. Secondary growth is via growth of vascular cambium and results in an increase in stem girth.

A cross-section of a stem reveals four main regions. The epidermis is the outer layer of the stem. Just in from the epidermis is the cortex. The cortex provides support and storage. The pith is a central core of parenchyma tissue in many stems. It is often crushed and destroyed by compression of secondary growth.

There are many different conformations of vascular tissues in plant stems. Vascular tissue is either scattered in the pith and cortex (monocots) or located between the pith and cortex (dicots).

Leaf arrangement on a stem is called phyllotaxy and it is very important in plant identification.

There are two very important distinctions between stem and root apices. One is that root apical meristems have a protective cap while stem apical meristems do not. The other is that stem apical meristems form lateral organs while root apices do not.

Leaves

True leaves are initiated via an apical meristem while seed leaves, or cotyledons, develop as part of the seed. True leaves consist of a lamina or leaf blade and a petiole. The lamina provides a large surface area for collecting light while the petiole holds the leaf erect and attaches it to the stem. A sessile leaf is a leaf without a petiole.

The thickened leaf base at the point of attachment to the stem is called the pulvinus. Stipules are small appendages often found at the base of the petiole. They are important in plant identification.

Leaves have three general tissue systems: (i) the epidermis; (ii) ground tissue; and (iii) vascular tissue.

The epidermis is the outermost layer with compactly arranged cells covered with a cuticle. There are stomata on one or both sides of the lamina.

Just interior to the epidermis is the ground tissue. The ground tissue is composed of spongy parenchyma and palisade parenchyma. The spongy parenchyma consists of loosely packed cells with significant intercellular spaces allowing for gas exchange. Spongy parenchyma cells are responsible for fixing carbon dioxide into carbohydrates during photosynthesis.

The palisade layer of parenchyma is one or more rows of tightly packed rectangular parenchyma

cells oriented to intercept maximum light. They are just interior to the upper epidermis and are loaded with chloroplasts. Sometimes the two cell types are not distinguishable and are collectively referred to as mesophyll.

The leaf vascular system is the network of xylem and phloem throughout the spongy parenchyma or mesophyll.

The abscission zone, located at the base of the petiole (or the lamina in sessile leaves) is a region of cells which upon exposure to specific environmental signals, dissolve the glue between cells allowing for leaf abscission.

Flowers

Flowers are modified leaves. Upon exposure to specific environmental signals such as daylength or temperature or at a specific point of plant development, shoot apical meristems start to form flowers rather than leaves. The process is extremely important in horticulture and will be covered in depth later (see Chapter 5, this volume).

Flowers are the reproductive shoot of an angiosperm (seed-bearing plant). They are composed of four whorls of modified leaves. Two sterile whorls include the sepals and the petals while two fertile whorls include the carpels and the stamen. The four whorls are attached to a common base or receptacle.

The outermost whorl is the calyx which is composed of individual sepals. The whorl just interior to the sepals is the corolla which is composed of petals. The calyx and corolla together comprise the perianth. The purpose of the perianth is to protect the fertile whorls and in some cases, to attract pollinators. If the petals and sepals cannot be distinguished, they are called tepals.

The two inner whorls include the stamen (collectively the androecium) which produce pollen grains followed by the carpels (collectively the gynoecium) which produce the eggs. Sexual reproduction results from pollination followed by fertilization and the subsequent production of seeds.

Besides being important in propagation, seeds are also usually required for proper fruit development. Seedless fruit are parthenocarpic. Parthenocarpic fruit develop from a flower in which the embryo has aborted.

The gynoecium is the megasporophyll and is composed of one or more carpels (modified leaves). The pistil in a flower is composed of one or more carpels and has three major parts: (i) the stigma; (ii) the style; and (iii) the ovary.

The androecium is the microsporophyll and includes the stamen. There are usually many stamens in a flower. Each stamen is composed of a filament holding an anther aloft. The anther is divided into pollen sacs which produce pollen grains. Each pollen grain in angiosperms consists of three cells surrounded by a thick protective wall. One cell is responsible for pollen tube growth down the style of the pistil, while the other two are generative nuclei. One sperm cell will unite with an egg cell to form the zygote while the other sperm cell fuses with the polar nuclei of the ovary to form the endosperm. This process is called double fertilization.

Fruit

Fruit is botanically defined as a ripened, mature ovary. A more acceptable definition considers that fruit may be derived from extracarpellary tissues as well as ovarian tissues which are united at maturity to form the harvested commodity. The simplest type of fruit is a single ripened carpel from a single ovary. An example is the peach. The pericarp is the ovary wall and it is divided into: (i) the exocarp (skin of the peach); (ii) the mesocarp (the flesh); and (iii) the endocarp (the pit). Inside the pericarp is one or more ripened ovules, or seeds.

Seeds

Most seeds are the result of double fertilization. The hard, outer coat is called the testa. Within the seed lies the embryo, a small diploid plant formed by fertilization of an egg by a pollen generative nucleus. The embryo has several major components including: (i) the cotyledons; (ii) the epicotyls; and (iii) the hypocotyl.

Cotyledons are also called seed leaves. They store energy needed for germination and become the first photosynthesizing organ of a seedling. Angiosperms have either one (monocots) or two (dicots) cotyledons. Gymnosperms may have many cotyledons. The epicotyl is the portion of the embryo above the cotyledons. The plumule is the meristematic apical tip of the embryo located at the apex of the epicotyl. The hypocotyl is the portion of the embryo below the cotyledons which terminates as the radicle, or embryonic root.

Plant Types

Humans love to classify things including plants. We classify them according to: (i) scientific nomenclature; (ii) growth cycle; (iii) stem growth; (iv) discipline; and (v) use.

Based on taxonomy – scientific classification

The most useful and widely accepted form of plant classification is that based on taxonomy. Plants are grouped together using morphological, anatomical, chemical and growth similarities. This type of classification is dynamic as classifications are revised as needed, based on new knowledge. There are seven main levels of taxonomic classification including: (i) kingdom; (ii) division; (iii) class; (iv) order; (v) family; (vi) genus; and (vii) species. Kingdom is the most inclusive while species is the least inclusive. Species is further divided into group or botanical variety and horticultural variety (or cultivar). A very good reference on the subject is Stuessy's (2008) text.

The practical use of taxonomic nomenclature ensures that we are talking about the same plant wherever we are on earth. We generally limit our plant name to one including genus, species, perhaps group, and finally cultivar. This text focuses on organisms in only one of the five kingdoms, and that is the plant kingdom or *Plantae*. All organisms in this kingdom have photosynthesis in common. Additionally, they are all eukaryotic, having differentiated cell types which contain vacuoles, chloroplasts and are surrounded by cell walls. They also reproduce either asexually or sexually via the alternation of generations. Within the plant kingdom there are vascular or non-vascular plants. Vascular plants reproduce via either seeds or spores.

This text also focuses on two seeded divisions within the vascular plants: (i) the *Pinophyta* (gymnosperms) where seeds are born on a cone; and (ii) the *Magnoliophyta* (angiosperms) where seeds reside in an ovary. Within the *Magnoliophyta*, we study two classes, the *Liliopsida* or monocots and the *Magnoliopsida* or dicots. Characteristics common to monocots include parallel leaf veins, flower parts in multiples of three, fibrous root systems, vascular bundles scattered in the pith and no annual growth rings. A monocot embryo has one cotyledon or seed leaf, hence the name. Characteristics common to dicots include a network of leaf veins, flower parts in multiples of four or five, tap roots with branches, and a vascular bundle in a single cylinder which results in the formation of growth rings. A dicot embryo has two cotyledons.

Within class we continue classifying into order, family, genus and species. Linnaeus developed the binomial system of plant nomenclature (Genus species) which is still used today. This system relies heavily on floral morphology.

Within Genus species we may identify the group (which used to be the botanical variety) and the cultivar which stands for cultivated variety. Horticulturists should make sure they consistently use the term cultivar rather than variety, since in almost all cases they are referring to the cultivar name and not the botanical variety.

The International Code of Nomenclature for Cultivated Plants

There are specific rules for writing the Latin binomial (McNeill *et al*., 2006). The first letter of the genus is always capitalized and the species is always lower case. Both genus and species are italicized or underlined, but not both, and the space between the genus and the species should not be underlined. The scientist responsible for naming the plant may be indicated with an initial (e.g. L., which stands for Linnaeus). Any revision to the original nomenclature is acknowledged after the original authority. The genus can stand alone but the species is never presented without the genus. The group may or may not be included, followed by the cultivar.

A good example of where the group is important in horticulture is in the family *Brassicaceae*. All of your childhood favorites are included in *Brassica oleracea*. To further categorize these vegetables correctly, we must include the botanical variety. When we need to use the botanical variety, it is because we have a group of related cultivars. We therefore indicate a group such as the Acephala Group, which includes the cultivars of kale. Note that neither the word 'group' nor the group name is italicized, and both are always capitalized. Thus we would present kale as *Brassica oleracea* Acephala Group. Note the cultivar has not been indicated.

When we want to identify a specific cultivar, we include it in the name according to the following example. Suppose we wanted to present the cabbage cultivar 'Copenhagen Market' in a report we were writing. We would indicate: *Brassica oleracea* (Capitata Group) 'Copenhagen Market'. Note the cultivar

name is not italicized, but rather, is enclosed in single quotes, and the group designation is enclosed in parentheses. Presentation of the cultivar using the cv. abbreviation and omitting the single quotes, such as: *Brassica oleracea* (Capitata Group) cv. Copenhagen Market is no longer acceptable.

To be complete, here's a list of your favorites (Fig. 1.2):

- *Brassica oleracea* Acephala Group – kale;
- *Brassica oleracea* Gemmifera Group – Brussels sprouts;
- *Brassica oleracea* Italica Group – broccoli;
- *Brassica oleracea* Botrytis Group – cauliflower;
- *Brassica oleracea* Caulorapa Group – kohlrabi; and
- *Brassica oleracea* Capitata Group – cabbage.

Classification by growth cycle

Plants are also often classified based on their growth cycle. In defining a particular growth cycle, we examine how long it takes for the plant to produce seeds from a seed. The time it takes is measured in growing seasons. We may also add descriptive terms to the definition to further categorize the plants in question.

Annual

Annuals are plants that produce seeds in one growing season and then die. Summer annuals complete their life cycle during the summer growing season (spring to fall) while winter annuals complete their life cycle over the winter growing season (fall to spring).

Biennial

Biennials require two growing seasons (usually summer) to produce seed. The two growing seasons are separated by a period of little to no growth while the plants are subjected to cold temperatures. This exposure to cold to induce or facilitate flowering and subsequent seed production is called vernalization. Biennial plants die after the second growing season.

Perennial

Perennial plants have the potential to produce seed every growing season after reaching a certain age (different for each species) and the plants may be very long lived. Perennial plants which go through a cyclical pattern of active growth followed by greatly reduced visible growth and shedding of

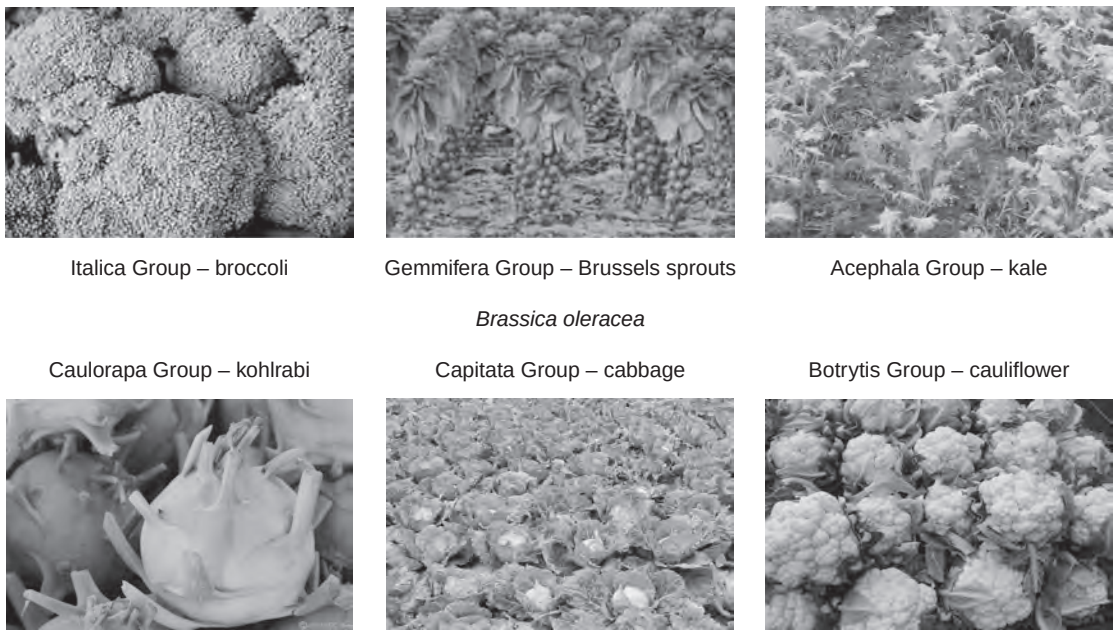

Fig. 1.2. Six major brassica commodities, all *Brassica oleracea.* Commodities are distinguished within species by different botanical groups.

leaves are called deciduous. Perennial plants which retain their leaves year-round, only shedding some of them over time, are called evergreen.

Classification by stem growth

We also classify plants according to the nature of their stem growth. Even though we like to list specific categories, there are many variations of the following plant types.

Herbs

Plants that produce soft, non-woody stems are called herbaceous. Herbaceous plants are often annuals. They are also usually relatively small in stature.

Shrubs

Plants with stems which become woody and have a number of co-dominant main stems arising at ground level, rather than one main trunk are called shrubs.

Trees

Trees are plants where the stem becomes woody and there is one main dominant trunk. Branching often occurs on the upper end of the main trunk

Classification by discipline

Plants can also be classified based upon which discipline of agriculture studies them. Generally we consider agronomic versus horticultural crops. Most crops are studied by either agronomists or horticulturists. A further characteristic which helps to place a crop under one or the other designation is the intensity of production. Horticultural crops often require intensive production practices while agronomic crops are usually less intensively grown. Sometimes the distinction is not really clear. For example, sweet corn (*Zea mays*) is a horticultural crop while field corn (*Zea mays*) is an agronomic crop. Thus their use helps to further classify them. Sweet corn is usually food (food for humans) while field corn is usually feed (food for animals) or grown for industrial use (starch, oil).

Classification by use

A plants ultimate use may determine its classification.

Ornamental

Plants used in the landscape or for other primarily aesthetic reasons are called ornamentals. Ornamentals are usually divided into landscape plants and floricultural crops.

Industrial

Many plants are grown for industrial uses, both agricultural and non-agricultural. They are all generally considered to be agronomic crops. Some non-agricultural uses might include drugs, latex, fiber, lumber or oil. Crops grown for use in agriculture, but not for direct human consumption are included in this category. Such crops would include hay, forage, silage, sugar, grains, cereal, pulses (grain legumes), green manure, cover crop, trap crop, and companion plantings.

Vegetable versus fruit

In horticulture, we often distinguish between fruits and vegetables. The botanical distinction is clear. However, the horticultural distinction of a fruit or vegetable may not be quite as clear. Any vegetative plant part that is consumed is always considered a vegetable. The distinction may be less clear when dealing with botanical fruit. If the botanical fruit (or fruit precursor, i.e. flower bud or flower) or product made from it is usually consumed as a major part of a meal, and is often savory rather than sweet, it is considered a vegetable. If it is consumed after the main meal or as a snack, and is usually sweet rather than savory, it is considered a fruit.

2 The Plant Hormones

We begin our exploration of plant-based physiology by studying the plant hormones. We will cover 'the big five' then take a look at some of the lesser known, yet equally important ones (Table 2.1; Fig. 2.1). We'll finish up by looking at the substances, both natural and synthetic, we use in horticultural production to modify commodity quality, quantity or both. Though we elucidate very specific roles for each hormone, the net effect on plant growth and development is the sum of all their individual actions and their interactions with each other. It is a very complex and complicated subject, of which we only scratch the surface of understanding.

The Term 'Hormone'

There has always been somewhat of an argument among plant scientists as to whether or not the term 'hormone' is appropriate in our discussions of organic substances which affect plant growth and development. While many think the use of the term 'hormone' is completely acceptable, others consider the term 'plant growth regulator' or a variant thereof more appropriate. The controversy stems from the animal science definition of 'hormone'. In that description, a hormone is an organic substance synthesized in one tissue and transported to another where it elicits an effect on growth and/or development. The main difference in plants is that their 'hormones' can have an effect in the tissue or cell in which it was synthesized; plant hormones don't necessarily have to travel.

In this text, 'hormone' is defined as:

> A naturally occurring organic substance produced by the plant, which at very low concentrations, controls plant growth and development through effects on cell division, elongation and differentiation either in the tissue of synthesis or elsewhere in the organism via long distance transport.

In general there are two classes of plant hormones: (i) inhibitors; and (ii) promoters. Some hormones promote certain plant processes while others inhibit different aspects of plant growth and development. Additionally, some hormones inhibit a process at one stage of development in a plant's life while promoting the same process at another stage. We will look at each specific hormone and answer the following questions about each:

1. Where is it synthesized?
2. What tissue(s) does it affect?
3. What does it do?
4. How does it do it?
5. Why is it important (or how can we use it in horticulture)?

We will not explore each hormone's history or biochemical synthesis to any great extent. The reader is encouraged to review the excellent reference *Plant Hormones: Biosynthesis, Signal Transduction, Action!* by Davies (2004a) for a good discussion of such matters. We will emphasize the basics for each hormone and illustrate horticultural uses of each or related substances. Please note that the description of horticultural uses for any of the hormones discussed does not imply a legal label for use in any country or any endorsement by the author for its use. Please consult local experts for regulations on specific uses in your area.

The Big Five

Auxins

Description

An auxin is any organic substance that promotes cell elongation in tissue segments when applied at low concentrations (Davies, 2004b).

Discovery and nomenclature

The major plant based auxin, indole-3-acetic acid (IAA), was discovered as the substance responsible

Table 2.1. The major plant hormones, their site of biosynthesis, mode of translocation and general function(s) in horticultural physiology.

Hormone	Primary site of biosynthesis	Primary mode of translocation	Primary function(s)
Auxin	Young meristematic tissue	Mass flow in phloem; polar transport	Cell elongation; vascular differentiation; root initiation; apical dominance; stimulates ethylene production
Cytokinin	Root tips; developing seeds	Xylem	Stimulates cell division; overcomes apical dominance; stimulates leaf blade growth; stimulates cell expansion
Gibberellin	Root and shoot apical meristems; young leaves; young fruits; developing seeds	Often synthesized at site of action; phloem; xylem; cell to cell	Stimulates stem elongation; replaces vernalization requirement of some long-day plants; affects floral sex expression; stimulates hydrolases in some germinating seeds; inhibits leaf senescence; inhibits root growth
Ethylene	All living plant tissue	Diffusion	Promotes fruit ripening, senescence and abscission; promotes leaf abscission; promotes (*Ananas*) or delays (*Prunus*) flowering; promotes the production of female flowers; induces epinasty
Abscissic acid	Mature leaves and roots; developing seeds	Mostly phloem	Induces stomatal closure; induces cessation of embryo growth in developing seeds; induces storage of seed proteins and development of desiccation tolerance in seeds
Florigen	Leaf phloem	Phloem	Induces the transition of meristems from vegetative to reproductive
Brassinosteroids	Pollen, seeds and young vegetative tissue	Synthesized at site of action	Promotes organ elongation; inhibits root formation and growth; induces xylem differentiation; stimulates seed germination
Jasmonates	All living plant tissues; leaves; young developing fruit; cotyledons of germinated seeds	Synthesized at site of action; phloem; xylem	Induce tendril coiling; inhibits general stem and root growth, photosynthesis, and seed germination; induces the production of storage proteins in tubers, bulbs and seeds; induces plant defense responses to insect and pathogen attack; increases production of secondary metabolites with a role in plant defenses
Polyamines	All living plant cells especially actively dividing ones	Phloem; xylem	Enhances cell division; prevents mitotic senescence; delays leaf senescence; may help regulate flowering; inhibits ripening and senescence
Salicylic acid	Leaves	Mostly phloem	Signal in thermogenic plants; signaling hormone in plant resistance to pathogens; may be a signaling molecule for flowering

for coleoptile bending towards a light source. The general term 'auxins' is used to refer to the group of substances which have auxin-like properties or elicit auxin-like responses when applied to growing plants.

There are four naturally occurring auxins in plants: (i) IAA; (ii) indole-3-butyric acid (IBA); (iii) phenylacetic acid (PAA); and (iv) 4-chloroindole-3-acetic acid (4-CI-IAA). IAA and IBA are the most widely known of the four. IBA is readily converted into IAA, thus there is some argument that it is a precursor to IAA rather than an individually isolated auxin. The other two auxins, 4-CI-IAA and PAA are recent discoveries, thus have been found in relatively few species. They too may be precursors of IAA (Normanly *et al*., 2004).

The synthetic auxins include IBA, napthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid

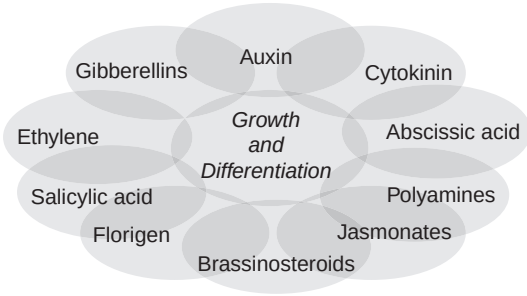

Fig. 2.1. The plant hormones.

(2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-methyl-4-chlorophenoxyacetic acid (MCPA).

Production

Auxin synthesis occurs in young meristematic tissues (Normanly *et al*., 2004). This includes the apical meristem of both the root and the shoot, developing embryos, young fruit, and young, rapidly growing leaves.

Transport

While auxin transport is generally described as basipetal, this is not entirely accurate. Basipetal movement occurs from either shoot or root apices towards the juncture of the root and stem. While auxin is synthesized in root apices, little if any moves towards the shoot. For the most part, auxin movement is from the shoot tip towards the root tip.

Auxin transport in cells and tissues is strongly polar, meaning that there is a very discernible gradient in auxin concentration. This gradient gives the 'signal' generated by the auxin direction. In fact, auxin itself may be responsible for developing and maintaining polarity in cells and the plant as a whole.

There are two modes of auxin transport in plants: (i) mass flow; and (ii) polar (Morris *et al*., 2004b). Auxin transport via mass flow occurs through the phloem from photosynthetic sources (leaves) to photosynthetic sinks (fruits, roots, and meristematic regions). Xylem is not involved in auxin transport via mass flow. Young leaves act as photosynthetic sinks early in their development and have a particularly high auxin content due to synthesis of auxin by the young leaf and auxin transported to them from other leaves via mass flow.

The second mechanism of auxin transport, polar transport, is much slower. In this system, auxin is moved from cell to cell from the shoot tip to the root tip. Movement is mediated by membrane-associated protein carriers and requires metabolic energy. Movement is through cambial cells and their derivatives, including developing xylem and phloem cells.

The two transport systems are not independent. Auxin does not enter the mass flow system from the polar system but auxin does enter the polar system from the mass flow system. This transfer from the phloem into the polar system occurs mostly in young tissues in the shoot apex. Movement via mass flow is easy to visualize as auxin going 'along for the ride' with photosynthates from the source to the sink. Polar movement is a little more complex.

Polar transport involves the active efflux of auxin from one cell into the apoplast followed by active uptake (influx) by an adjacent cell. This must occur along a cellular continuum from the shoot tip to the root tip and may occur against a gradient. It does not require cytoplasmic continuity between adjacent cells. In order to understand how these factors fit together, we need to examine polar transport at the cellular level.

Auxin is an acid. Non-dissociated auxin molecules in the apoplast (pH ~5.5) move across the plasma membrane into the cytoplasm (pH 7.0) and quickly dissociate. The auxin dissociates due to pH, thus as more molecules enter the cytoplasm, they dissociate as long as the pH of the cytoplasm remains around 7. The higher pH in the cytoplasm is maintained via an energy-requiring plasma-membrane-bound proton pump that pumps protons out of the cell which in turn acidifies the apoplast. Since auxin molecules continue to dissociate once in the cytoplasm, a concentration gradient for non-dissociated auxin exists across the plasma membrane and auxin can continue to move into the cell via diffusion. Auxin anions cannot freely cross the plasma membrane, thus they remain in the cytoplasm. The only way for them to move out of the cytoplasm is via an efflux carrier molecule in the plasma membrane. Auxin anions may also enter the cytoplasm from the apoplast via influx carriers which transport auxin anions across the plasma membrane into the cytoplasm (Hagen *et al*., 2004). The efflux of auxin molecules from the cytoplasm regulates the degree of the polar gradient.

Effects

CELL AND STEM ELONGATION There are many known effects of auxin on plant growth and development. One of the most widely studied effects is increased stem elongation associated with increased cellular elongation (Cleland, 2004). Auxin induces cell elongation within 10 min of application to stem or coleoptile segments resulting in a five to tenfold increase in length. While auxin stimulates stem elongation, it inhibits the elongation of root cells, particularly in the central zone of elongation. Just as pH influences auxin transport from cell to cell, pH is also important in eliciting the initial cell wall loosening associated with cell elongation due to auxin application. Auxin induces the active transport of protons out of the cytoplasm into the apoplast via an ATP-requiring proton pump. The decreased pH induces the manufacture and activity of wall-loosening enzymes. For prolonged stem elongation via cell elongation induced by auxin, the osmotic potential of the cytoplasm must remain low via import of solutes into the cell. This maintained low osmotic potential ensures the high turgor needed for cell expansion. Additionally, the cell wall must remain susceptible to auxin-induced wall loosening.

TROPIC RESPONSES Auxin is responsible for two very important plant tropic responses: (i) phototropism; and (ii) gravitropism. Both of these responses are discussed in Chapter 4, this volume. Briefly, auxin accumulates on the stem or root side away from the stimulus (the dark side for phototropic responses and the 'up' side for gravitropic responses), greater cell elongation occurs on that side as a direct result of auxin action, and the stem or root bends towards the stimulus.

CELL DIVISION AND VASCULAR DIFFERENTIATION Auxin stimulates cell division in the cambium and also regulates the differentiation of phloem and xylem tissue (Aloni, 2004).

ROOT INITIATION Auxin stimulates root initiation on stem cuttings and is used extensively for propagating hard-to-root species. While auxin stimulates root formation, it inhibits root elongation via stimulation of ethylene production.

LATERAL SHOOT INHIBITION (APICAL DOMINANCE) Auxin inhibits lateral shoot production in many species by inhibiting lateral bud growth. This is known as apical dominance. Growers often remove the apical meristem by pinching to encourage branching, resulting in a green thumb and a bushy plant. Additionally, auxin inhibits adventitious bud growth.

STIMULATION OF ETHYLENE PRODUCTION Auxin promotes flowering in bromeliads by inducing ethylene production. Additionally, auxin-stimulated ethylene stimulates the production of female flowers in cucurbits and other dioecious plants.

FRUIT SET AND GROWTH Auxin promotes fruit set in tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), holly (*Ilex* spp.), okra (*Abelmoschus esculentus*), figs (*Ficus* spp.), and cucurbits. Auxin also promotes fruit growth. In most fruit it is an absolute requirement. Fruit lacking a full complement of seeds are often small and misshapen compared with their fully seeded counterpart. Some species produce parthenocarpic fruit, fruit with no seeds. In these species, auxin is produced by the fruit tissue itself, rather than by the developing embryo in the seed.

FRUIT AND LEAF ABSCISSION Auxin can have completely opposite effects depending on the stage of plant development at the time of application. A great example of this is observed in apple (*Malus domestica*) production. Auxin applied to apple trees in the spring promotes abscission of many, but not all of the flowers. The idea is to remove excessive numbers of flowers to reduce competition among them for photosynthates. This process is called thinning. If not enough flowers or young fruit are removed during the thinning process, fruit quality including size will suffer severely, resulting in many small fruit. Excessive thinning would result in unacceptably low yields. About 3.5 months later, a similar auxin application results in the retention of fruit on the tree, exactly opposite to the earlier effect. Apples have a tendency to abscise prematurely, resulting in reduced yield and bruising of fallen fruit. The timing and rate of application for both uses must be carefully controlled to achieve the desired results.

Auxin helps regulate the formation of leaf abscission zones as well. When the relative level of auxin in a leaf versus its parent stem favors the leaf, an abscission zone does not develop. Once the auxin level in the leaf falls below that of the stem, the abscission zone forms and the leaf is ultimately shed.

Horticultural utilization

The naturally occurring auxin IAA is: (i) very expensive to extract from plant tissue; (ii) subject to degradation by enzymes; and (iii) extremely sensitive to degradation by light. Therefore horticultural uses of auxins involve the synthetic auxins, primarily IBA, NAA and 2,4-D.

One of the most prevalent uses of synthetic auxins is the use of 2,4-D, as a selective broadleaf herbicide. Even though it is considered a selective broadleaf herbicide, grasses may be affected as well. It is normally applied to foliage and is translocated in the phloem to multiple sinks in the plant. It kills plants by inducing uncontrolled growth, resulting in severe stem twisting and leaf malformation and overall plant dysfunction. Death of the affected plant is slow. Many grapes are extremely sensitive (parts per billion) to drift from 2,4-D applications, thus extreme care must be used when using it around vineyards.

Vegetative propagation via stem cuttings is enhanced using auxins. Exposing the basal end of a stem cutting to a powder (usually talcum powder) containing IBA or soaking the basal end in a solution of IBA in water often enhances root initiation of the cutting. The length for soaking and the concentration of IBA needed to enhance rooting varies with species.

Foliar application of NAA at 10 ppm is often used for thinning apples. Later in the season, foliar auxin is again utilized, however, then it is used to prevent pre-harvest drop.

Foliar application of NAA induces ethylene production which subsequently induces floral induction in pineapple.

Fruit set of tomato in the absence of adequate pollination and fertilization can be enhanced with auxin application.

Cytokinins

Description

Cytokinins are substances which promote cytokinesis in tissue culture (in the presence of auxin) (Davies, 2004b).

Discovery and nomenclature

Kinetin, the first known cytokinin, was discovered in autoclaved herring sperm DNA. It has never been isolated from plant tissue. The first plant-based cytokinin was *trans*-zeatin which was isolated from corn (maize) endosperm in the 1960s (Miller *et al*., 1955; Miller, 1961; Letham, 1963). Since then a number of cytokinins have been isolated from plant tissue. The naturally occurring cytokinins are mostly based on zeatin. They are often classified into active, storage or translocated forms. The two major synthetic cytokinins are benzyladenine (BA) and kinetin. Both compounds are stable as they are not subject to degradation caused by zeatin-metabolizing enzymes.

Production

Cytokinins are produced primarily in root tips and developing seeds. However, any tissue with a high rate of cell division may produce cytokinins (Sakakibara, 2004).

Transport

Cytokinins are transported primarily in the xylem. Phloem sap also contains cytokinins, but at much lower levels than xylem sap.

Effects

CELL DIVISION Cytokinins stimulate cell division in tissue culture callus, but growth is limited if auxins are absent. In the presence of auxin and cytokinins, callus growth is robust and undifferentiated. If the balance of auxin to cytokinin in tissue culture is shifted towards cytokinin, shoot production is favored. If it is shifted towards auxin, root production is favored. Thus by altering the levels of these hormones in the growth media, callus tissue can be stimulated to form shoots followed by roots, eventually leading to clonal production of new plantlets.

Stimulation of cell division also occurs in crown gall tumors as a result of infection by the bacteria *Agrobacterium tumefaciens*. The tumors are masses of undifferentiated cells often observed at the base or crown of a plant caused by an overproduction of cytokinins and auxins. This overproduction is caused by a small loop of non-chromosomal DNA called a plasmid carried by the bacteria. The plasmid carries the genetic code for auxin, zeatin and opine production. Opines are nitrogen-containing molecules that serve as food for the bacteria. When the plant is infected with the bacteria, the plasmid DNA is incorporated into the plant's genome and

the overproduction of auxin and cytokinin begins. As a result, a mass of non-differentiated tissue forms on the stem.

Control of cell division by cytokinins is through their control of the movement of cells out of the G2 phase of mitosis, regulated by cyclin-dependent protein kinases (CDKs) and cyclins (Roef and Onckelen, 2004). CDKs are enzymes responsible for adding a phosphate to a protein. Cyclins are proteins which regulate the CDK enzymes. The combination of CDKs and cyclins provide a regulatory switch for controlling the cell cycle.

OVERCOMING APICAL DOMINANCE Cytokinins stimulate the release of lateral buds from apical dominance imposed by auxin from the apical bud. In addition, cytokinin application promotes the development of dormant buds.

STIMULATION OF LEAF BLADE GROWTH Cytokinins stimulate leaf blade growth via cell enlargement. The balance between leaf area and root volume is regulated via this mechanism. A larger leaf area can be supported by a greater number of roots. With more roots, more cytokinins are produced and transported to the leaves, resulting in leaf blade enlargement. If the number of roots is limited, less leaf blade enlargement will occur. In addition, cytokinins promote chloroplast and chlorophyll development and delay leaf senescence. Cytokinins applied to a leaf will induce sink-like activity in the leaf, thereby promoting leaf longevity by directing nutrients to the leaf.

CELL EXPANSION Cytokinins can also stimulate cell expansion by increasing the plasticity of the cell wall. This increased cell wall plasticity is not related to the increased plasticity stimulated via acidification with auxin application.

Horticultural utilization

Use of cytokinins in production horticulture is widespread. Commercial formulations of 6-benzyladenine (BA) are available from a number of manufacturers, and are often a mixture of BA and gibberellins.

Fruit size and weight is enhanced via fruit thinning with an application of BA in apple (*M. domestica*) and pear (*Pyrus* spp.) production. Thinning also promotes annual bearing in normally biennial bearing cultivars. When environmental conditions are not favorable for producing the typical prominent calyx lobes in 'Red Delicious' apple cultivars, application of a mixture of BA and gibberellic acid (GA) promotes such a shape.

Enhanced lateral branching in nursery stock of apple (*M. domestica*), pear (*Pyrus* spp.) and cherry (*Prunus* spp.) can be induced with BA. This lateral branching accelerates the formation of a well-branched tree once transplanted in the orchard. Increased branch angles are also induced with nursery application of BA. Lateral branching of terminal growth in the orchard can be enhanced as well.

Application of a mixture of BA and GA to lily (*Lilium* spp.) bulbs delays senescence of lower leaves and open flowers. If gibberellin-inhibiting growth regulators have caused retarded growth in bedding-plant plug production, treatment with a BA/gibberellin combination helps overcome the growth inhibition.

A similar BA/gibberellin combination spray of poinsettia (*Euphorbia pulcherrima*) plants can promote overall vegetative growth if it is applied before short-day induction of flowering. A late-season spray can be used to promote bract expansion.

Another synthetic cytokinin, forchlorfenuron increases berry size and uniformity in table grape (*Vitus* spp.) and kiwifruit (*Actinidia* spp.) leading to increased yield. Fruit firmness and delayed ambering of green table grapes is also achieved.

Gibberellins

Description

The gibberellins are substances which promote stem, root and fruit growth (Davies, 2004b). There are over 110 different molecular forms of gibberellin and they are identified as gibberellic acid (GA)1, GA2, . . . GA*n*. Although they are similar in structure, they are all very different in their biological activity. Only about 30% of the known gibberellins are physiologically active with the rest likely to be breakdown products or precursors. GA1 is the most biologically active GA and GA3 is the easiest and least expensive GA to extract from fungal cultures for commercial use. All higher plants are assumed to contain GA in one form or another and they occur in free or conjugated forms.

Discovery and nomenclature

A devastating disease of rice causes an excessive elongation of seedling shoots leading to lodging of the

entire plant and ultimate crop losses. This disease is called the 'foolish seedling' disease and is caused by the fungus *Gibberella fujikuro* (Kurosawa, 1926; Brian *et al*., 1954). The substance (GA3) responsible for the pale, spindly weak growth of shoots was isolated from cultures of the fungus and given the name gibberellin. Since its discovery, over 110 forms of GA have been isolated from plant tissues.

Production

Growing meristematic tissue including root and shoot apical cells, young leaves, young fruits, and developing seeds (especially the endosperm) produce GA (Sponsel and Hedden, 2004).

Gibberellins are produced by fungal cultures and purified to obtain GA3 for use in commercial horticulture. GA3 is used because this is the only gibberellin obtainable in commercial quantities. A more expensive mixture of GA4 and GA7 is now available for specific uses.

Transport

GAs are often synthesized in the tissue where they elicit their effect. Transport occurs primarily in the phloem, but may also occur in the xylem and from cell to cell.

There is often a gradient of GA in plants that supports a high level of GA at the shoot apex which decreases basipetally. GA levels are often high in root tips as well. It appears that GAs are transported from the shoots to the roots via the phloem. There they may be transformed into a different form of GA and translocated back to the shoot via the xylem.

Effects

STEM ELONGATION GA induces extensive stem growth in many rosette plants and dwarf mutants. Stem elongation is a combination of enhanced cell elongation followed by increased cell division in the sub-apical meristem. Work with rice has indicated that the GA-induced cell elongation precedes GA-induced cell division. GA first enhances cell elongation via an increase in cell wall elasticity by a mechanism that is unknown and different than that caused by auxin. When cells are large enough, they transition from the G1 to the S phase of the cell cycle (Sun, 2004).

FLOWERING In some long-day plants requiring vernalization (exposure to a cold treatment) where flowering is preceded by stem elongation, the long-day and/or cold treatment can be replaced with GA. Examples of plants with either of these two requirements include celery (*Apium graveolens*), sugarbeet (*Beta vulgaris*), foxglove (*Digitalis purpurea*), and flowering stock (*Matthiola incana*). It seems that in these cases of enhanced flowering, it is the stem elongation prerequisite for flowering that is enhanced by the GA, not flower formation per se. In most other long-day plants whose stems do not elongate before flowering and in all short-day plants, GA treatment does not stimulate flowering.

In some specific long-day plants where stem elongation does not precede flowering (lettuce (*Lactuca sativa*), radish (*Raphanus sativus*) and spinach (*Spinacia oleracea*)) the long-day stimulus can be replaced with GA. Additionally, GA promotes flowering in coneflower (*Asteraceae*), petunia (*Petunia* × *hybrida*) and Douglas fir (*Pseudotsuga* spp.) and enhances flower bud formation in cherries (*Prunus* spp.). GA treatment can also substitute for chilling in artichoke (*Cynara cardunculus*), resulting in earlier flower production.

Besides stimulating flowering in certain species, GA can also affect the floral sex expression. Treatment with GA promotes the production of male flowers in papaya (*Carica papaya*), cucumber (*Cucumis sativus*), and some melons (*Cucumis melo*). The production of female flowers is promoted in begonia (*Begonia* spp.), chinese chestnut (*Castanea mollissima*), and castor bean (*Ricinus communis*).

GAs generally stimulate pollen germination and subsequent pollen tube growth down the style leading to fertilization. In some species such as tomato (*S. lycopersicum*), grape (*Vitis* spp.), stone fruit (*Prunus* spp.), apples (*M. domestica*) and pears (*Pyrus* spp.), GA enhances fruit set above that which normally occurs. Improvement of fruit set in apples and pears is particularly important when adverse weather conditions during pollination results in poor natural fruit set. Parthenocarpy (fruit production without fertilization of the egg) in apples, pumpkin (*Cucurbita* spp.), and eggplant (*S. melongena*) may also be stimulated with GA.

When GAs are applied in the fall to plants which initiated flower buds the previous summer, normal spring flowering is inhibited. Plants in which this response has been observed include apple, grape,

and peach. This phenomenon has been investigated for its potential to avoid frost damage to flowers in the spring by delaying bloom.

SEED GERMINATION Certain seeds have a specific long-day photoperiodic requirement for germination. The long-day requirement (and thus a short night) leads to high levels of phytocrhome far red (P_{fr}) which stimulates germination. GA treatment can substitute for the long-day requirement and therefore the high P_{fr} requirement.

In grains such as barley (*Hordeum vulgare*), GA controls the formation and activity of hydrolases which metabolize starch into maltose, a key sugar in the brewing process. GA also improves the nutrient supply to the embryo during germination. Specifically, GA is produced by the cotyledon (scutellum) of the embryo and stimulates amylase production, which converts starch into simple sugars. These simple sugars are absorbed by the scutellum and translocated to the embryo for growth (Woodger *et al*., 2004).

GROWTH INHIBITION Gibberellins inhibit both leaf senescense and root growth. In addition, GA treatment reduces rind senescence in oranges (*Citrus* spp.) which permits longer 'on-tree' storage. This helps extend the marketing season.

Horticultural utilization

Enhanced stem elongation associated with GA is widely utilized in commercial horticulture. Celery (*A. graveolens*) and rhubarb (*Rheum* spp.) stalks are elongated by GA sprays. In the production of Thompson seedless grapes (*Vitis vinifera*) individual berries elongate in response to GA application. In addition, the entire cluster elongates, resulting in a larger cluster with larger fruit.

Parsley (*Petroselinum hortense*) yield is increased with GA. In roses (*Rosa* spp.), stems are elongated using GA. Tree geraniums (*Pelargonium* spp.) are created by stimulating stem elongation with GA combined with careful pruning.

Sugarcane (*Saccharum* spp.) yield is increased with GA treatment via two mechanisms. Stem elongation is enhanced concomitant with increased sucrose production such that the sugar concentration in the stem is not diluted by the elongation induced by the GA.

There are a number of chemicals which inhibit gibberellin synthesis that are used extensively in ornamental horticuluture including Phosphon D, CCC (cycocel), Amo1618, Ancymidol (A-rest), paclobutrazol and B-Nine (alar). These types of substances will be discussed later in this chapter under 'Synthetic Plant Growth Regulators and Their Uses in Horticulture'.

Ethylene

Description

Ethylene is a single substance which exists in nature as a gas. It is the only hydrocarbon with a pronounced effect on plant growth and development (Adams and Yang, 1981; Davies, 2004b; Pech *et al*., 2004).

Discovery and nomenclature

Ethylene in illuminating gas used to light street lamps in the late 1800s was identified as the substance responsible for premature defoliation and stunted growth observed in plants located around street gas lights, especially leaky ones (Doubt, 1917). Ethylene is a single substance therefore there is no need for a discussion of nomenclature.

Production

Ethylene is produced in all plant tissue, often as a response to stress such as drought, flooding, mechanical pressure, injury, or infection. Ethylene production is often stimulated by auxin. Meristematic regions and senescing tissues, especially fruit, are rich sources of ethylene gas. In general, nodes produce more ethylene than internodes.

Ethylene is produced from methionine, an amino acid (Pech *et al*., 2004). ATP reacts with methionine to form *S*-adenosyl-L-methionine (SAM), a carrier molecule for methionine. SAM is converted to 1-aminocyclopropane-1-carboxylic-acid (ACC) by the enzyme ACC synthase. It is the activity of this enzyme which regulates ethylene production in plant tissues. ACC synthase is coded by a multi-gene family and is found in the cytosol. It is induced by auxins, flower senescence, fruit ripening, wounding, chilling injury, flooding, drought, and ethylene. This stimulation of ACC synthase by ethylene makes ethylene production autocatalytic. This is important. Once ethylene production is induced in ripening fruit,

more and more ethylene is produced, further accelerating ripening and senescence.

There are a number of known ethylene inhibitors. Silver ions, CO_2 and $KMnO_4$ inhibit ethylene at the site of activity by interfering with binding of ethylene to receptors. Aminovinylglycine (AVG) and aminooxyacetic acid (AOA) inhibit ACC synthase, thereby regulating ethylene production rather than reception.

Transport

Ethylene travels through the plant via diffusion from cell to cell. Since it is produced in all tissues, its transport is not normally necessary for an effect to be realized. Ethylene also diffuses out of plant tissue and into the atmosphere, thus elevated levels within plant tissues depends on continued production.

Effects

Ethylene has many known effects on plant growth and development. Much of our knowledge of ethylene activity comes from exposing plant tissues or organs with ethylene in enclosed containers. With the development of ethephon (2-chloroethylphosphonic acid) ethylene application is greatly simplified. Ethephon is applied as an aqueous spray and is absorbed into plant tissues. There it decomposes to release ethylene gas, chloride, and phosphate ions.

FRUIT RIPENING, SENESCENCE, AND ABSCISSION Ethylene promotes fruit ripening, senescence, and abscission.

LEAF ABSCISSION Ethylene promotes leaf abscission. Even though ethylene is generally universally present in plant tissues, auxins produced by the leaf or fruit reduce the sensitivity to ethylene of abscission zone cells in leaves and fruit. As auxin levels decline, abscission zone cells become more sensitive to ethylene and the production of cellulases increases. Cellulases weaken wall connections and the weight of the leaf or fruit is enough to allow the leaf or fruit to fall off the plant.

FLOWERING Ethylene promotes flowering in bromeliads, which includes pineapple (*Ananas comosus*).

On the other end of the spectrum, ethylene can delay flowering in some *Prunus* species. When ethylene applied as ethephon to peach (*Prunus persica*) or cherry (*Prunus avium*) in the fall at approximately 50% leaf fall, flowering is delayed the following spring by as much as 14 days. This fall application of ethylene also increases the cold hardiness of peach flower buds by reducing the size and water content and increasing the sugar content of the pistil. Even though pistil size is reduced with the ethylene application, final fruit size is not affected. In addition, the dehardening response often observed in *Prunus* flower buds upon exposure to warm temperatures after the chilling requirement has been fulfilled is greatly reduced.

Ethylene applied as ethephon promotes femaleness in many crops.

EPINASTY A common malady of houseplants is overwatering. Excessive watering leads to ethylene production by the plant which induces epinasty, a downward bending or drooping of leaves. Caretakers often see this drooping as a sign of water stress and proceed to water the plant even more. This only exacerbates the problem.

THE ETHYLENE TRIPLE RESPONSE A widely used plant indicator of ethylene is the triple response of pea (*Pisum sativum*). Pea seedlings treated with ethylene show a triple response to the gas proportional to the level of exposure. The three responses include: (i) greatly shortened internodes; (ii) increased stem diameter; and (iii) a lack of the normal gravitropic response (stems growing up and roots growing down). Furthermore, leaves fail to expand and the shoot apex remains hooked.

THIGMOMORPHOGENESIS Responses of plants to touch, thigmomorphogenesis, are usually attributable to ethylene action.

ROOT GROWTH Ethylene stimulates auxin biosynthesis and transport towards the root elongation zone where it leads to the inhibition of cell elongation, thus resulting in reduced root growth.

SEED GERMINATION AND BUD SPROUTING The stimulation of germination in cereals and peanuts as well as sprouting in potatoes and bulb crops is also attributed to ethylene action.

FRUIT RIPENING Controlling the level of ethylene in produce storage facilities is crucial in regulating postharvest physiology of horticultural crops.

This aspect of ethylene action will be discussed in Chapter 17, this volume. Ethylene enhances ripening of fruit that are harvested mature but not ripe, for example, banana (*Musa* spp.).

LATEX FLOW Ethephon enhances latex flow in rubber trees (*Hevea brasiliensis*) by delaying the healing of tapping wounds. Tapping wounds are the cuts made to allow latex to flow from the plant.

STEM ELONGATION AND THICKENING Ethylene inhibits stem elongation in terrestrial plants with a concomitant increase in stem thickness. Stem thickening is attributed to reorientation of microtubules and microfibrils from mostly transverse to oblique. In contrast, many semi-aquatic plant stems elongate rapidly upon submergence and its concomitant accumulation of ethylene in the underwater tissue. The stem elongation is a result of increased sensitivity to GA induced by the ethylene. The ethylene also causes a decline in abscisic acid, which is a potent inhibitor of GA. Thus the increased response to GA may actually be due to the reduction in abscisic acid caused by increased ethylene production.

Horticultural utilization

Ethylene is used as a harvest aid to promote fruit abscission in cherry (*Prunus* spp.), apple (*M. domestica*), citrus (*Citrus* spp.), nuts and olives (*Olea europaea*). In addition, ethephon is used to enhance uniform coloration and ripening of tomatoes (*S. lycopersicum*) for mechanical harvesting.

In commercial pineapple (*A. comosus*) fields, natural production of ethylene by the plant is stimulated with applications of auxins or with an application of ethylene via ethephon. Plants are treated when they are 6 months old when they are at the 30 leaf stage, about 3 months prior to their natural time of bloom. Induction of flowering with ethylene promotes uniform flowering, fruiting, and cropping.

Ethylene also promotes female flower production in cucurbits (cucumber, squash, melons (*Cucurbita* spp.)) increasing the number of fruits produced per plant.

Abscisic acid

Description

Abscisic acid (ABA) is often described as an inhibitor. This is unfortunate as ABA promotes several physiological components of plant growth and development.

Discovery

Abscisic acid was initially discovered as an inhibitor of oat (*Avena sativa*) coleoptile growth. Soon after, a 'similar' substance named abscission II was described that stimulated the abscission of cotton (*Gossypium* spp.) bolls. Another substance produced in sycamore (*Plantanus* spp.) leaves that promoted bud dormancy was discovered by another group who named the substance dormin. All three groups were actually working with the same substance and in 1967 the name abscisic acid was given to this new plant hormone. Unfortunately, the name really does not describe the function, since ABA has little involvement in the control of fruit and leaf abscission and relatively little involvement in the control of bud dormancy.

Nomenclature

ABA is a single substance, rather than a group of related substances as in the auxins, GAs and cytokinins. There are a number of forms of the molecule, however, the most prevalent form in plants is (+)-2-*cis*, 4-*trans* abscisic acid, more simply known as abscisic acid or ABA.

Production

ABA is primarily synthesized in mature leaves and roots in response to water stress (Schwartz and Zeevaart, 2004). It may also be synthesized in just about all other plant tissues. Developing seeds are also rich in ABA which is either synthesized *in situ* or imported from leaves or roots.

It is mostly synthesized from carotenoids. Extremely large changes in endogenous levels of ABA can occur rapidly in response to stress and ABA levels are regulated at a number of levels. ABA levels can vary via degradation, compartmentalization, transport to other tissues, conjugation with a sugar, or conversion into phaseic or dihydrophaseic acid.

Transport

Long-distance transport is mostly in the phloem and to a lesser degree, in the xylem. At the cellular level, ABA exists in different forms depending on pH.

At a neutral pH, ABA exists in a dissociated state. At a mildly acidic pH (5.0–6.5) ABA is mostly undissociated. At more acidic pHs (<5.0) ABA is mostly in a protonated form. The undissociated and protonated forms diffuse freely across cell membranes but the anionic form requires active uptake via carriers.

Effects

Although ABA is often classified as a growth inhibitor, this is unfortunate since ABA promotes certain aspects of plant development such as seed maturation, dormancy, and plant survival under certain stresses. As with all hormones, the response to exogenous ABA depends on the tissue and stage of development. ABA applied to hypocotyls, epicotyls, coleoptiles, and leaves generally results in growth inhibition. Application to roots can either inhibit or promote growth. In germinating seeds and excised embryos, ABA inhibits further embryo growth and development. ABA is also known to prevent vivipary, the uninterrupted development of embryos without a dormant period. ABA seems to have very limited involvement in abscission and senescence of leaves and fruits. ABA counteracts the effect of gibberellin on α-amylase synthesis in germinating cereal grains. ABA inhibits cell division in fronds and roots of *Lemna minor* perhaps by promoting the production of a protein which inhibits kinase activity in cyclin–CDK complexes in the cell cycle. There are limited reports of ABA promoting cold hardiness, but much of the work in this area of ABA involvement in plant growth and development has been inconsistent.

PLANT STRESS AND CROSS-PROTECTION ABA increases during times of plant stress, providing protection from the stress via various mechanisms. In this sense, ABA acts as a promotor, promoting plant survival. During drought stress, ABA levels in leaves rise dramatically, causing closure of stomata and the production of proteins known to protect membranes and other cellular structures during dehydration. Proteins also reduce the osmotic potential of the cytoplasm, further preserving what water is left in the cell. Similar proteins are also important in protecting seeds from the severe dehydration which accompanies their maturation. In addition, synthesis of seed storage proteins is enhanced with ABA.

The general response of increased synthesis of specific proteins during many different types of stresses such as stress brought about by salt, heat, and pathogen attack, has led to the description of ABA as the stress hormone in plants. When a plant elicits a protective response to one form of stress, protection from other potential stresses is also incurred. This is known as 'cross-protection'.

WATER STRESS RESPONSES Water stress can be induced by drought, salinity, and freezing temperatures. Independent of the mode of stress induction leading to cellular dehydration, water stress induces a rapid increase in ABA synthesis. The increase in tissue ABA content is transient, since upon removal of the stress, ABA synthesis decreases and levels decline accordingly. Morover, even if the stress is not removed, ABA levels begin to decline. Since ABA levels do not remain high concomitant with the stress, ABA must be a signal for cellular stress-coping mechanisms to occur.

ABA application can mimic drought stress responses by plants, namely reduced bud and shoot growth, stomatal closure, and reduced photosynthesis. Proteins which form in response to the stress can be induced with an application of ABA. ABA-deficient mutants of *Arabidopsis* and tomato (*S. lycopersicum*) cannot tolerate drought stress (their stomata won't close), however, application of ABA to mutant plants under stress elicits responses similar to those of non-mutants under stress.

Dehydration stress induces ABA synthesis which induces the formation of specific stress proteins. But how does a plant sense the dehydration stress to induce ABA synthesis? Several things happen at the cellular level concomitant with dehydration. There is: (i) a decrease in cellular water content along with an increase in solute concentration; (ii) a decrease in osmotic potential of the cytoplasm; and (iii) a reduction in cellular turgor. The component most highly related to ABA synthesis is the loss of cellular turgor and perturbations of the plasma membrane associated with it. How this triggers ABA synthesis is not known.

The initial stimulus of dehydration is probably perceived first by the roots and the signal, in the form of ABA, is sent to the leaves to induce stomatal closure (Fig. 2.2). Stomatal closing has been more closely associated with soil water potential rather than leaf water potential. Lowered soil water potential causes a significant increase in xylem sap ABA concentration.

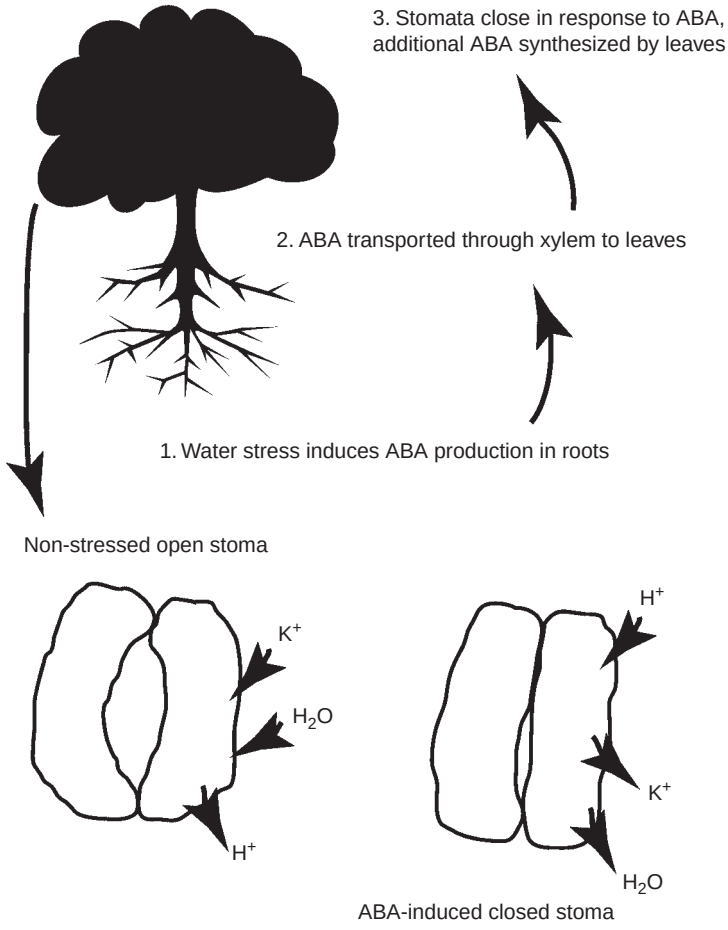

Fig. 2.2. The water-stress-induced response in plants regulated by abscisic acid (ABA).

The concentration of ABA in the xylem is more closely correlated to closing of stomata than either leaf water potential or leaf ABA concentration. While there is convincing evidence for the transfer of the stress signal from the roots to the leaves via ABA in the xylem, one cannot say conclusively that all of the ABA originated in the roots. Some ABA from the shoot may also enter the xylem.

Wherever the ABA originates, it eventually accumulates in the apoplast of leaf epidermal guard cells. Guard cells have no plasmodesmatal connections with any adjacent epidermal or mesophyll cells. Any signal must be transmitted via the apoplast. The ABA receptor at the guard cell remains unidentified. It does, however, seem likely to be a receptor on the plasma membrane. Whether it is located on the apoplastic or symplastic side is still not known. Direct injections of ABA into the cytosol induce responses associated with increased ABA levels, thus at least some of the reception occurs on the symplastic side of the membrane. However, the stomatal response to ABA is more closely related to apoplastic ABA levels, especially under mild to moderate stress conditions.

ABA inhibits stomatal opening and promotes stomatal closure via control of ion-mediated osmotic changes in the guard cells.

Stomatal opening can be triggered by blue and red light, low internal CO_2 concentrations, high atmospheric humidity and the fungal toxin fusicoccin. These stimuli activate ATP-driven proton pumps on the plasma membrane that force H^+ out of the cytosol and into the apoplast. This reduces

the apoplastic pH and increases the transmembrane potential to a more negative value. This in turn stimulates K^+ uptake through voltage-regulated channels in the membrane. Guard cells also take up Cl^- ions, but the mechanism is not known. In addition malate ions act as counterions for the K^+.

During the dehydration stress the apoplastic ABA signal inhibits the ATP-driven proton pumps and induces a decrease in cytosolic pH, an increase in cytosolic Ca^{2+} concentration, inhibits the inward influx channels of K^+ and depolarizes the plasma membrane. The increased Ca^{2+} levels in the cytosol inhibits inward movement of K^+ and at the same time promotes the efflux of K^+ out of the cytosol. The depolarization of the membrane also causes K^+ ions to leave the cytosol. The overall loss of K^+ from the cytosol is concomitant with an efflux of water from the guard cell and the resulting decrease in guard cell turgor allows the stomatal pore to close.

Once the dehydration stress is over, the ATP pumps resume their work and the apoplast pH drops and ABA re-enters the symplast for storage or metabolism. Stomatal opening ensues and apoplastic ABA levels return to pre-stress levels awaiting the next dehydration event, where they will once again increase and the process starts all over again.

The previous description is of the rapid, almost immediate short-term responses induced by ABA under drought stress. Longer-term drought stress incorporates changes in gene expression induced by ABA. In particular, proteins involved in K^+ influx decrease and enzymes involved in carbon metabolism show differential expression after longer-term (2–4 days) drought stress.

SEED MATURATION AND DORMANCY A second major function of ABA is its involvement in seed maturation and dormancy. In general, seed development is often separated into three stages: (i) morphogenesis; (ii) cell enlargement with reserve accumulation; and (iii) developmental arrest and desiccation. The latter two are often combined into a collective stage called maturation. One of the first steps in this process is the cessation of embryo growth due to cell cycle arrest at the G1/S transition which is concomitant with a sharp increase in seed ABA levels. The second part of maturation is the accumulation of storage reserves accompanying cell enlargement and dehydration. It is during this stage that ABA levels in the seed reach their highest. The final stage includes the development of desiccation tolerance, water loss, and a decrease in ABA levels. ABA has a limited role in the induction and maintenance of dormancy in some seeds, but does not seem to play a major role in the regulation of 'true' dormancy that is only broken with low temperature or light.

Horticultural utilization

There are no practical uses of ABA in horticulture. ABA is very expensive to synthesize. Additionally, it degrades rapidly in the light. Analogs of ABA have been developed in the quest to find a chemical which could confer drought resistance. Increases in cold hardiness have been associated with ABA and ABA analog application, however, consistent positive results are lacking.

Some Other Growth Regulating Substances

A number of other substances having growth regulator properties have been identified in plants. Some of the more popular ones are presented in the following section. Whether or not these substances are "officially" classified as plant hormones is academic. They have profound effects on plant growth and development and possess many of the characteristics of the classic hormones, thus they are important.

Florigen

A long popular theory in plant science is that flowering is triggered by the relative levels of the five major plant hormones that are modified by changing environmental conditions. When environmental conditions are favorable for flowering in sensitive species, there is a balance of hormones in the plant which promotes this transition. This idea is rather complicated as simultaneous measurement of the five major hormones is difficult and different environmental conditions can induce flowering in the same species.

Many plant scientists have also sought a single substance, 'florigen', that is responsible for the transition from the vegetative to the sexually reproductive state in plants. The existence for such a substance was controversial, however, molecular-level research with *Arabidopsis* led to the discovery of florigen (Zeevaart, 2008). *Arabidopsis* is a model plant which is induced to flower with long-day exposure. There are two genes responsible for flowering in *Arabidopsis*, the CONSTANS (CO) gene and the FLOWERING LOCUS (FL) gene.

The CO gene encodes a protein that when exposed to long days induces the transcription of the second gene (FT) in the phloem of leaves. Florigen is the FT gene product, a mobile protein of approximately 20 kDa which is transported through the phloem to a receptive meristem causing flowering. While all other hormones are extractable substances which can be applied to plants and induce specific responses, the proteinaceous nature of florigen precludes such extraction and application. However, molecular techniques have allowed transplanting the FT gene to other species. Overexpression of the FT gene has led to flowering under non-inductive conditions in many species. In addition, overexpression of the FT gene greatly reduced the juvenile period in perennial species.

Florigen is required for flowering in all plants and is not species specific. Florigen is produced in leaves of photoperiodically sensitive species under the control of phytochrome. In day-neutral species, the level of florigen, and therefore flowering, is not regulated by daylength.

Brassinosteroids

The term brassinosteroid (BR) refers to the naturally occurring steroids found in plants that elicit growth responses in nanomolar or micromolar doses. A substance first isolated from rape (*Brassica napus*) pollen was given the name brassin and later identified as brassinolide, the first plant steroid with growth regulating activity. Brassinolide caused extreme elongation of pinto bean internodes. Since brassinolide's discovery, more than 50 steroids have been isolated from many different species, thus BRs are likely to be ubiquitous in the plant kingdom. The two most common steroids in plants are brassinolide and castasterone, a brassinolide precursor.

BRs are produced in almost all plant tissues, but especially in seeds, pollen, and young vegetative tissue (Choe, 2004). Their presence in roots has not been confirmed, and exogenous application of BR inhibits root formation and growth. BRs promote stem, petiole, and peduncle elongation in dicots and promotes elongation of colepotiles and mesocotyls in monocots. BRs often promote organ elongation by promoting cell wall loosening and subsequent cell elongation but not cell division. In some species, BRs will stimulate cell division in the presence of auxin and cytokinin. They do so by regulating the production of the cyclin 3 protein which is important in the release of cells from the G1 to the S stage of the cell cycle.

BR-deficient mutants of *Arabidopsis*, tomato and pea all exhibit severe lack of stem elongation. Exogenous BR application fully restores normal stem elongation in such mutants, indicating that BRs are essential for normal stem elongation.

BRs are also involved in plant responses to light. BR-deficient mutants of tomato and *Arabidopsis* grown in the dark often lack an apical hook, have expanded cotyledons, form true leaves, and express genes associated with photosynthesis and anthocyanin production. All of these attributes are normally observed in light-grown seedlings! Thus a lack of BR simulates the presence of light.

BRs also stimulate tracheid formation in differentiating xylem tissue. In BR-deficient mutants, too much phloem and not enough xylem are differentiated. Additionally, deficient mutants are male sterile, and plant senescence is delayed. Pollen tube growth is promoted by BRs. Application of BR enhances ethylene production and subsequently promotes epinasty, senescence, and leaf abscision.

BRs can reverse an ABA-induced seed dormancy while stimulating germination.

Although there are many reports of enhanced yield, vigor and stress tolerance with the application of exogenous BRs, they are extremely inconsistent.

Jasmonates

Jasmonates are a group of oxylipins (oxygenated fatty acid derivatives) which include jasmonic acid and methyl jasmonate (Howe, 2004). Methyl jasmonate is a fragrant volatile component of the essential oils of rosemary (*Rosmarinus officinalis*), jasmine (*Jasminum*), and many other flowers.

In early studies in the 1980s, both jasmonic acid and methyl jasmonate were observed to retard root and coleoptile growth and promote leaf senescence.

Jasmonates are found in many higher plants, green and red algae, and in some fungi. They are produced in all plant tissues, especially upon wounding. Leaves, young developing fruit, and cotyledons of germinated seeds are particularly rich in jasmonates. Even though jasmonic acid is more prevalent in plant tissue than methyl jasmonate, plant responses to exogenous applications are more readily observed when methyl jasmonate rather than jasmonic acid is applied.

Jasmonates can induce tendril coiling, much like that caused by ethylene. Jasmonates inhibit general stem and root growth, photosynthesis, and seed germination. Jasmonates induce the production of storage proteins in tubers, bulbs, and seeds. Jasmonates are important for promoting male reproductive development in *Arabidopsis*. They also stimulate potato (*Solanum tuberosum*) tuberization, fruit ripening, leaf and flower senescence, and abscission.

Jasmonates have been identified as the major compounds responsible for inducing plant defense responses to insect and pathogen attack. When a plant is mechanically wounded or an insect chews on a leaf, an 18 amino acid protein called systemin is produced. Systemin is derived from a larger protein called prosystemin. Upon chewing, the gene for prosystemin production is activated in the vascular tissue and systemin is translocated in the phloem. Proteins associated with increased defense are synthesized in leaves near and far from the attack or injury as a result of the systemin signal.

In addition, oligosacharride fragments may be released from cell walls and the fragments may act as elicitors. Elicitors are compounds (proteins, peptides, lipids, and polysaccharides) of microbial origin which initiate a plant's defense response system. These elicitors may not travel far from the attack site, but can induce the gene for prosystemin which leads to higher systemin levels in the plant. The systemin or oligosacharride fragments may induce jasmonate production which can then induce the defense-related genes. Methyl jasmonate is volatile and may serve as an airborne signal to neighboring plants that an attack is underway.

Jasmonates also increase the production of secondary metabolites that play a role in plant defenses. The plant defense response may be direct or indirect. Direct regulation is accomplished by production of phytochemicals that negatively affect the plant attacker, its feeding, growth, or reproduction. For example, upon feeding by an attacking herbivore, a plant may produce proteinase inhibitors or polyphenoloxidases which reduce the digestibility of the plant tissue. Indirect regulation comes via an interaction of the host, the herbivore, and an enemy of the herbivore, such as a predator or a parasite. When caterpillars feed on certain plants, the plant responds by producing terpenoids in response to fatty acid amide elicitors in the secretions of the feeding caterpillar (Fig. 2.3). The terpenoids released by the plant allow caterpillar parasites, such as parasitic wasps, to locate the caterpillar.

Fig. 2.3. A tobacco hornworm (*Manduca sexta*) infested with a parasitic braconid wasp (*Cotesia congregata*). Upon feeding on the tomato plant (*Solanum lycopersicum*) fatty acid amide elicitors in the saliva of the hornworm induce the production of volatiles by the tomato which are detected by the wasp, allowing the wasp to find the hornworm and parasitize it. Note the elongated wasp larvae on the back of the hornworm.

Polyamines

Polyamines are strongly basic protein-based substances of low molecular weight that exist either free or bound in all plant cells (Ryan and Pearce, 2004). At a physiological pH, all polyamines are positively charged and bind strongly to negatively charged enzymes. When polyamines bind to enzymes, the enzyme's activity is altered. The main polyamines in plants include putrescine, spermadine, and spermine.

Polyamines should be considered hormones for a number of reasons: (i) they are present in all cells; (ii) they exert noticeable regulatory control over growth and development; and (iii) they are effective at micromolar concentrations.

Polyamines enhance cell division which is required for tuber formation in potatoes (*S. tuberosum*). Polyamine levels are very high in actively dividing cells and very low in cells that are not. Polyamines prevent mitotic senescence. In Jerusalem artichoke (*Helianthus tuberosus*) tubers, low polyamine levels are associated with low rates of cell division. Treatment with exogenous polyamine enhances cell division. Treatment with IAA also enhances cell division with an increase in polyamine levels.

Exogenous applications of polyamines delay leaf senescence by preventing chlorophyll loss, membrane peroxidation, and inhibiting RNase and

protease activity. In addition, polyamine levels are higher in green versus senescing leaves.

Polyamines are also implicated in regulating the flowering process. Polyamines accumulate in the shoot apex, buds, and flower parts of many plants. In *Arabidopsis*, polyamine levels are low in the rosette and bolt, but increase dramatically in the flowers. In tobacco, flowering of thin cell-layer explants can be regulated with spermidine. When the explants are programmed to flower, they are very high in spermidine. If spermidine production is inhibited with cyclohexamide, spermadine levels decrease with a concomitant decrease in flowering. If the inhibitor is removed, spermadine levels increase along with flowering. Cultures grown under conditions supporting vegetative growth will flower if treated with spermadine.

Pharbatis nil is a short-day plant that can be induced to flower with one long night. Under long-day conditions, application of putrescine will induce flowering.

In carrot (*Daucus carota*) and *Vigna* tissue culture, callus proliferation occurs if polyamine levels are low. If polyamine levels are increased, embryoid formation occurs. In tobacco (*Nicotiana tabacum*) tissue culture, the overproduction of spermidine leads to anther rather than ovary production.

Polyamines interact with other known plant hormones in a variety of ways. IBA induces root formation in mung bean (*Vigna radiata*) explants which is accompanied by a twofold increase in polyamine levels. If explants are treated with IBA and a polyamine inhibitor, fewer roots are initiated.

Parthenocarpic fruit induced with an application of auxin can be inhibited with the polyamine inhibitor difluoromethylornithine (DFMO). The inhibition of parthenocarpy with DFMO can be reversed with an application of putrescine.

Cytokinin induces cotyledon expansion in cucumbers (*C. sativus*) which is accompanied by increases in polyamine levels. In pea leaves, senescence is accompanied by a decrease in polyamines. Cytokinin-induced retardation of senescence is accompanied by a retardation in the decline of polyamine levels.

GAs are known to increase polyamine levels in plants. GA-induced dwarf pea (*P. sativum*) internode elongation is accompanied by an increase in polyamines. This elongation is primarily due to increased cell division rather than elongation. α-Amylase activity in germinating barley (*H. vulgare*) seeds is enhanced with GA application which is also accompanied by an increase in polyamine levels.

Ethylene and polyamines are antagonistic with respect to their effects on ripening in climacteric fruit and leaf senescence. Ethylene promotes ripening and senescence while polyamines inhibit both processes.

Salicylic acid

Salicylic acid (SA) is a phenolic plant hormone with roles in plant growth and development, photosynthesis, transpiration, ion uptake and transport (Delaney, 2004). It is most widely known for its roles in signaling for plant defense against pathogens, thermogenicity in plants, and flowering in certain species.

Willow (*Salix*) bark was known for its pain-relieving properties by the ancient Greeks and Native Americans. The active ingredient responsible for this pain-relieving attribute was isolated, identified and named salicylic acid in the early 1800s. Commercial production of synthetic SA began in Germany in the late 1800s and Aspirin, a trade name for acetylsalicylic acid, was introduced by the Bayer Company in 1898. Aspirin rapidly became one of world's best-selling drugs.

Salicylic or ortho-hydroxybenzoic acid belongs to a diverse group of plant phenolics and is widely distributed in plants. The highest levels are found in the inflorescences of thermogenic plants and in plants infected with necrosis-inducing pathogens.

SA and flowering

The first indications that SA was involved in flowering were observed in tobacco (*N. tabacum*) tissue culture, but these observations never attracted much attention since many compounds were known to induce flower bud formation in tobacco tissue culture.

The hypothesis that SA was directly involved in flowering came about after the observation that some factor was transmitted by aphids feeding on short-day flowering *Xanthium strumarum* plants to vegetative plants growing under long days. Honeydew extracts from aphids feeding on flowering *Xanthium* could induce flowering in the long-day plant *Lemna gibba* strain G3 under non-inductive conditions. The flower-inducing substance in the honeydew was identified as SA. This flower-inducing effect has been demonstrated in

other short- and long-day species in the family *Lemnaceae*, in the ornamental orchid *Oncidium*, in *Impatiens balsamina, Arabidopsis thaliana*, and in *Pisita stratiotes* L.

Even though SA was identified in the flower-inducing honeydew, SA itself is not the endogenous flowering regulator, but rather a signaling molecule. SA does not induce flowering in *Xanthium*, members of the *Lemnaceae*, and other plants. Additionally, the levels of SA in honeydew from flowering and vegetative plants do not differ.

Thermogenic plants

Heat production (thermogenicity) occurs in some plant species. It occurs during flowering of plants in the genus *Arum* and occurs in the male reproductive structures of cycads and inflorescences of some angiosperm species belonging to the families *Annonaceae, Araceae, Aristolochiaceae, Cyclanthaceae, Nymphaeaceae*, and *Palmae*.

This heat generation, which can be as much as 14°C above ambient temperature, is associated with an increase in cyanide-insensitive, non-phosphorylating electron transport which is unique in plant mitochondria. Oxygen consumption during this heat generation is equal to that of a hummingbird in flight. Besides the activation of the alternative oxidase pathway, the heat generation requires activation of the glycolytic and Krebs' enzymes.

In the voodoo lily (*Sauromatum guttatum* Schott) the heat generation facilitates the release of foul-smelling amines and indoles to attract insect pollinators. In the 1930s it was suggested that some substance was produced in the plant which induced this incredible burst of heat. It was given the name kcalorigen. It wasn't until 1987 that kcalorigen was identified as SA. The production of SA in thermogenesis regulation is controlled by photoperiod.

SA and disease resistance

SA is the signaling hormone in plant resistance to pathogens and plays a key role in regulating systemic acquired resistance (SAR) and the hypersensitive reaction (HR) in disease-resistant plants.

SAR is where a pathogenic attack on one part of a plant induces resistance to pathogens in other parts of the plant. The intra-plant signal for the development of SAR is SA. When SA is converted by the plant to volatile methyl salicylate, the signal can become interplant, where a pathogen attack on one plant can be perceived by another plant where SAR can be induced in the perceiving plant. The protection afforded by SAR may last several weeks and may offer protection against pathogens not related to the inducing organism.

HR is a response to pathogen attack seen in some disease-resistant plants. In plants which have HR, a necrotic lesion develops around the initial point of pathogen attack via death of cells in the necrotic area. This HR may lead to SAR.

As part of the physiological development of both HR and SAR a number of low molecular weight PR (pathogenesis related) proteins are often produced by the plant. Proof that these proteins are directly related to HR and SAR is lacking, however, their presence during acquisition of disease resistance is well known. Some additional evidence that these proteins are involved includes their induction by application of SA even in the absence of pathogens.

Tobacco (*N. tabacum* cultivar 'Xanthi-nc') has an 'N' gene which confers an HR response to tobacco mosaic virus (TMV) which includes production of PR-1 proteins. Leaf treatment with SA induces the same PR-1 proteins and protection from TMV increases with increasing SA concentration. TMV-susceptible *N. tabacum* has the recessive 'n' allele of the 'N' gene and TMV does not trigger PR proteins and the plant exhibits the mosaic pattern in young leaves. Aspirin (acetylsalicylic acid) application induces PR proteins and the mosaic spread is reduced. SA may induce other mechanisms of resistance other than PR proteins. If SA in fact does induce disease resistance, imagine the possibilities for modern agriculture.

Synthetic Plant Growth Regulators and Their Uses in Horticulture

There are many synthetic chemicals acting as plant growth regulators which are used to improve the quality or yield of horticultural crops or to make their management easier. It would be impossible to cover all of the chemicals used worldwide for management of horticultural crops. As an example of the ways in which plant growth regulators might assist production of horticultural crops, plant growth regulator use in apples (*M. domestica*) is closely examined. A general discussion of plant growth regulator uses in ornamental and turf horticulture follows. While

specific chemicals are discussed, recommendations are left to local experts. In addition, mention of a trade name does not imply an endorsement of any product.

Apple crop management

Apples production relies heavily on the use of plant growth regulators, both naturally occurring and synthetics. One important distinction that apples bring to the table compared to the other crops discussed is that apples are consumed by humans. Therefore the health ramifications of ingesting the discussed substances are considered during their development. Products undergo enormous testing before they are labeled for use.

Growth regulators used to improve crop management

Apple nursery stock and non-bearing trees are often treated with Promalin or Perlan (*N*-(phenylmethyl)-1H-purine 6-amine, also known as 6-benzyladenine, a synthetic cytokinin) combined with Gibberellins A4A7 (GA4+7) to increase lateral bud break and shoot growth as well as improving (widening) branch angles. Benzyladenine enhances lateral bud break since it is a cytokinin while the GAs promote the elongation of shoots which grow from the broken buds. Promalin can also be used to improve branching of terminal shoot growth on bearing trees.

Many cultivars and rootstocks of apples are prone to suckering. Suckers emerge from below the ground and can be controlled with NAA, or its ethyl ester, ethyl 1-naphthaleneacetate (Tre-Hold sprout inhibitor). Watersprouts, vigorous upright shoots, often originate around pruning cuts and can be controlled with the same two chemicals.

Apple fruit is normally thinned in the early stages of development to increase fruit size and reduce crop load to encourage annual fruiting. Thinning 'Golden Delicious' fruit can be accomplished by applying the synthetic auxin NAA around 2–3 weeks after full bloom. The auxin application results in abscission of weaker fruit, resulting in effective thinning. If the NAA is applied too early, fruit will not abscise and thinning will not be achieved. This phenomenon highlights the enormous effect the stage of development can have on efficacy of growth regulator application.

In some apple cultivars, NAA is not effective as a thinner. Non-spur 'Red Delicious' and 'Rome' are thinned with the insecticide carbaryl (Sevin). Carbaryl is not used to thin 'Golden Delicious' as it tends to cause russetting in that cultivar. Extreme care must be used with carbaryl as it is extremely toxic to honeybees.

To thin spur strains of 'Red Delicious' a combination of carbaryl (Sevin) + NAA is applied when fruit are small (average fruit diameter of 9–11 mm). Larger fruit (12–15 mm) can be thinned with a combination of carbaryl and ethephon. Application of carbaryl + NAA at this larger fruit size (12–15 mm) to 'Red Delicious' can cause "nubbins" and half-grown fruit which stick to the tree through harvest. Again, this emphasizes the effect developmental stage can have on response to growth regulators.

To remove all the fruit from apple trees if they are not large enough to bear a crop, a combination of NAA + carbaryl (Sevin) + ethephon (Ethrel) can be used. Besides completely thinning the tree, this combination will suppress vegetative growth for a while.

Thinning cultivars such as 'Gala', 'Fuji', and 'Spur Red Delicious' is accomplished using N-(phenylmethyl) -1H-purine-6-amine (Exilis, MaxCel) + carbaryl (Sevin). 'Stayman', 'Rome', 'McIntosh', 'Jonathan', or 'Gala' can be thinned with carbaryl (Sevin), or NAA, or carbaryl (Sevin) + NAA.

Apples often suffer from biennial bearing. Return bloom can be encouraged without a thinning effect if NAA is applied biweekly for 2 months beginning 6 weeks after petal fall.

The growth regulator prohexadione-calcium (Apogee) can be used to reduce vegetative growth and later-season tree canopy volume and density. This improves pesticide penetration and efficiency.

Apple fruit tend to fall off the tree before they are ready for harvest. Pre-harvest drop can be prevented with sprays of NAA. Applying a low rate of NAA before fruit loosening begins (preloading) is much more effective in preventing premature drop than using a higher rate when the fruit begins to loosen. Preloading may also increase return bloom of 'Golden Delicious' and 'Red Delicious' cultivars.

Pre-harvest treatment to improve quality

A number of measures can be taken before harvest to improve the quality of apple fruit; many of these measures include growth regulators. Spraying with

aminoethoxyvinylglycine (AVG) (ReTain) will delay pre-harvest fruit drop and maturity which allows time for an increase in fruit size.

Some apple cultivars such as 'Delicious', 'Gala' and 'Ginger Gold', may not produce fruit with a desirable shape or fruit weight. An improvement in fruit quality can be achieved with an application of Promalin or Perlan, the same chemicals used to induce better lateral branching in the nursery. The combination of cytokinin and GAs in the Promalin or Perlan increases fruit size via increased cell division induced by the cytokinin and increased cell size induced by the GAs. In addition, the GAs reduce fruit russetting.

Some apple cultivars, particularly 'Golden Delicious', are susceptible to russet formation, a corky epidermal growth around lenticels on the fruit skin. To reduce or prevent russetting, GA4+7 can be applied to trees at petal fall, followed by repeat applications 10, 20, and 30 days after petal fall. GAs also help prevent fruit cracking, a particular problem in 'Stayman' apples, if applied every 3 weeks beginning about 3 weeks before anticipated cracking.

Postharvest treatment to improve storage

Scald is a postharvest disorder in apples that appears after about 3 months of storage. It appears as discoloration on the fruit's surface within about 3–7 days after removal from cold storage, greatly reducing their market value. Diphenylamine (DPA) can be applied as a dip or spray to harvested fruit to reduce the occurrence of scald.

Another treatment that reduces scald as well as maintaining fruit firmness and acidity is treatment with 1-methylcyclopropene (MCP) (SmartFresh). Treatment involves introducing MCP into the atmosphere of the storage container or facility for 24 h. Following treatment, fruit can be held in regular cold storage.

Growth control in greenhouse crops

With most greenhouse grown crops, a standard plant size and form is desired for each particular crop. To achieve this 'ideal' plant, many compounds are available for use in greenhouse production. Both floricultural and vegetable crops are greenhouse grown, however, the vast majority of growth regulators are used on floricultural crops. Few plant growth regulators are labeled for greenhouse vegetable production.

The most common growth regulators used in the greenhouse industry include: ancymidol, daminozide, paclobutrazol, chlomequat chloride, uniconazole, benzyladenine, GA3, GA4+7 + benzyladenine, ethephon, and flurprimidol.

Daminozide and chlormequat chloride are usually applied as foliar sprays to provide short-term inhibition of stem elongation. They are often used on plug crops which only need a slight reduction in stem height.

Ancymidol, flurprimidol, paclobutrazol, and uniconazole are applied as foliar sprays, soil drenches, or liner dips to reduce stem elongation. The differences among the products are the strength of their stem elongation reduction and the length of their effectiveness. Ancymidol has the weakest and most short-lived effect while uniconazole has the strongest and longest lasting effect; those in between have moderate effects on stem elongation and medium longevity of action. Uniconazole-P is the first and only plant growth retardant approved for use in greenhouse production of vegetable crop transplants of eggplant (*S. melongena*), pepper (*C. annuum*), groundcherry (*Physalis* spp.), pepino (*Solanum muricatum*), tomatillo (*Physalis philadelphica*) and tomato (*S. lycopersicum*).

GA3 and GA4+7 + benzyladenine are applied as foliar sprays and promote stem elongation and reduce yellowing of older leaves in crops such as lily (*Lilium* spp.) or geranium (*Pelargonium* spp.). They can also be applied to counteract the over application of a stem elongation inhibitor.

Ethephon is applied as a foliar spray and releases ethylene gas which inhibits stem elongation, causes flower bud and flower abortion, and often increases branching. It is sometimes used to maintain crops in a vegetative state as stock plants for propagation.

Benzyladenine applied without GA stimulates lateral branching and sometimes flowering.

Growth control in woody ornamentals

A widely used plant growth regulator in woody ornamental horticulture is auxin. Auxins are available in a number of forms as a rooting stimulant (K-IAA, IBA, K-IBA, K-NAA, IBA + NAA, IBA, naphthaleneacetomide (NAM), IBA + NAA).

Compact plants are produced in the nursery using daminozide, dikegulac sodium and paclobutrazol.

Lateral branching can be stimulated with application of dikegulac sodium and lateral shoot growth of azalea (*Rhododendron* spp.), *Cotoneaster, Juniperus*, and *Taxus* can be promoted with methyl decanoate/octanoate.

Dikegulac sodium suppresses flowering and fruit formation. Ethephon is used to reduce or eliminate undesirable fruit development on many ornamental trees and shrubs. To retard regrowth of most trees, shrubs, and vines, chlorflurenol is often utilized. Maleic hydrazide is often used to retard regrowth of most trees, shrubs, and ivy (*Hedera* spp.).

Turf management

One of the main expenses in turf maintenance is mowing. Three main groups of chemical are used in turfgrass management to retard general plant growth and thus reduce the amount of mowing necessary. These three groups are: (i) herbicides; (ii) Class I inhibitors; and (iii) Class II inhibitors.

Herbicides

Herbicides can be used at low rates to inhibit plant growth. Herbicides commonly used for this purpose include glyphosate, chlorsulfuron, imazameth, imazethapyr + imazapyr, metsulfuron, sethoxydim and sulfometuron. These herbicides act at the cellular level through varying mechanisms.

Class I – cell division inhibitors

The group of chemicals called Class I retardants act by inhibiting cell division. These chemicals include amidochlor, chlorflurenol, maleic hydrazide, and mefluidide. These substances are foliarly applied. Their main effects are retarded leaf growth and inhibited seed head formation. They can be phytotoxic, and as such, are often used on 'lower value' or 'low maintenance' turf.

Class II – GA biosynthesis inhibitors

Class II retardants are GA biosynthesis inhibitors. They are applied foliarly and reduce leaf growth but do not retard seed head formation. One chemical is trinexapac-ethyl which is absorbed through the leaves. Others, absorbed through the roots, include flurprimidol, and paclobutrazol.

Miscellaneous plant growth regulator uses in horticulture

Desiccants for aiding harvest

A number of crops are much more easily harvested if vegetative tissues are desiccated prior to harvest. Some crops benefiting from pre-harvest desiccation include chili peppers (*C. annuum*) for drying, dry beans (*Phaseolus* spp.), potatoes (*S. tuberosum*), sunflower (*Helianthus annuum*) and tomatoes (*S. lycopersicum*) for processing. A number of chemicals are available for use as desiccants including sodium chlorate (Chlorate, Defol), paraquat (Firestorm, Gramoxone Inteon), diquat (Reglone), and glufosinate (Rely).

Pre-harvest treatment to aid harvesting

Tomatoes grown for processing are mechanically harvested all at once, requiring uniform ripening among the fruit. Ethephon (Ethrel) is an ethylene-releasing growth regulator which can be foliarly applied to induce uniform ripening.

Postharvest treatment to prolong quality

In order to prevent sprouting in storage (Fig. 2.4) and the greatly reduced storage life associated with sprouting, onions (*Allium cepa*) and potatoes (*S. tuberosum*) are often treated with a potassium salt of maleic hydrazide. Maleic hydrazide works by non-selectively inhibiting cell division.

Fig. 2.4. Potatoes (*Solanum tuberosum*) sprouting just 3 weeks after harvest. To prevent sprouting, potatoes are often treated with the growth inhibitor maleic hydrazide. Maleic hydrazide prevents sprouting by non-selectively inhibiting cell division.

3 Growth, Development, and Plant Movement

What is Growth?

Growth is an irreversible increase in the number and/or size of cells in a living organism. Depending on the tissue and stage of development, growth characteristics may change over time. For a while growth may rely on cell division, while later in development it may primarily rely on cell enlargement.

For example, consider the fruit of the peach (*Prunus persica*) tree. During the initial stages of fruit growth just after pollination and fertilization, much of the fruit growth is occurring inside the developing seed. Much of this early seed growth is due to an increase in the number of cells. If you observed the fruit on the tree during this time, you would not think that much growth was occurring, since the small green fruit does not enlarge very much. Later on when the flesh of the fruit begins to enlarge, growth is due to an increase in both cell number and cell size. Even later, during final fruit growth as the peach nears maturity, most of the growth is due to an increase in cell size.

A key part of the definition of growth is that of irreversibility. Once the number of cells in an organism increases, their number normally does not decrease. It is true that some cells may get sloughed off as they senesce. Even though certain plant tissues may appear to become smaller and shrink, making it appear as if growth has been reversed, this decrease in size is usually due to a reduction of water content or a depletion of stored food reserves. Consider a potato (*Solanum tuberosum*) that begins to shrivel and become smaller. This decrease in size is due to a depletion of starch in the tuber as it is metabolized during senescence.

Senescence can be envisioned as the opposite of growth; a decrease in size due to a decrease in cell size (due to water loss and utilization of cell reserves), number (due to cell death), or both. This is a great oversimplification of the senescence process. Many other things happen during senescence, and we'll address those in Chapter 16, this volume.

Growth curves

How do we measure growth? Most often growth is quantified by observing the increase over time of one or more selected variables. Growth is often measured at different levels. At the cellular level we might look at an increase in cell number, cell diameter, cell volume, etc. Or we might be interested on a whole plant or tissue level. We could measure shoot length, stem diameter, fruit volume, fruit weight, etc.

Once we have a series of measurements over time, we can create a picture of the growth over time of our organism. This graphical representation of growth over time is called a growth curve. We study growth curves so that we might better study the effects of various production practices or environmental factors on plant growth.

While entire textbooks have been devoted to the growth curves exhibited by plants, we will limit our discussion to several of the most common curves studied.

Exponential

Exponential growth is characterized by an increasing rate of growth over time. For example, supposed we were measuring the growth rate of a tomato (*Solanum lycopersicum*) seedling by measuring the length of the shoot (in millimeters) from the cotyledons to the shoot apex (the epicotyl). We begin measuring once the cotyledons are fully exposed during germination and continue for 14 days. We collect the data presented in Table 3.1.

Plotting the data, we produce the curve presented in Fig. 3.1.

Notice that the growth is not simply a straight line where the shoot length is increasing slowly over time. One of the major characteristics of the exponential growth curve is that growth increases at a faster and faster rate as time passes. Growth almost seems out of control, however, some of the factors

responsible for this incredible growth soon become limiting. Additionally, factors within the plant itself, such as age, change and soon become limiting.

We usually want to describe the growth we have measured with a mathematical equation. This is called modeling. We could determine the type of curve we are dealing with by looking in a textbook of plant growth curves and finding the one that most closely mirrors ours. We could also fit various models to our data using computer software and select the model which best fits our data. It is obvious just by looking at our plot that a straight line would not be the best fit for our data (Fig. 3.2).

Table 3.1. Epicotyl length of tomato seedlings from the day of complete cotyledon expansion until 14 days later.

Day	Epicotyl length (mm)	Day	Epicotyl length (mm)
1	2	8	54
2	3	9	87
3	5	10	140
4	8	11	224
5	13	12	359
6	21	13	575
7	34	14	920

The straight line implies that growth increases by a fixed amount for each unit increase in time. In our example, this would imply that shoot length increased by a constant amount each day. Our graph certainly shows that shoot length increases, on average, by a greater amount each day. You could verify this by calculating the increase in epicotyl length per day for the data in Table 3.1 to produce Table 3.2.

The growth rate itself is growing over time. By how much? The percentage increase in epicotyl length per day is presented in Table 3.3.

At first, the epicotyl length increases by relatively small amounts, 1 mm the first day, 2 mm the second, 3 mm the third, 5 mm the fourth, etc. Note that by the tenth or eleventh day, epicotyl length increases by 53 mm, then 84 mm and the increase continues to increase after that. This is exponential growth. If we calculate the increase as a percentage (as seen in the fourth column), we see that the epicotyl length is increasing by about 60% each day. This is classic exponential growth.

A property of exponential growth is that whenever the independent variable (time) increases by one unit, the dependent variable (epicotyl length) increases by a set percentage, in this case 60%.

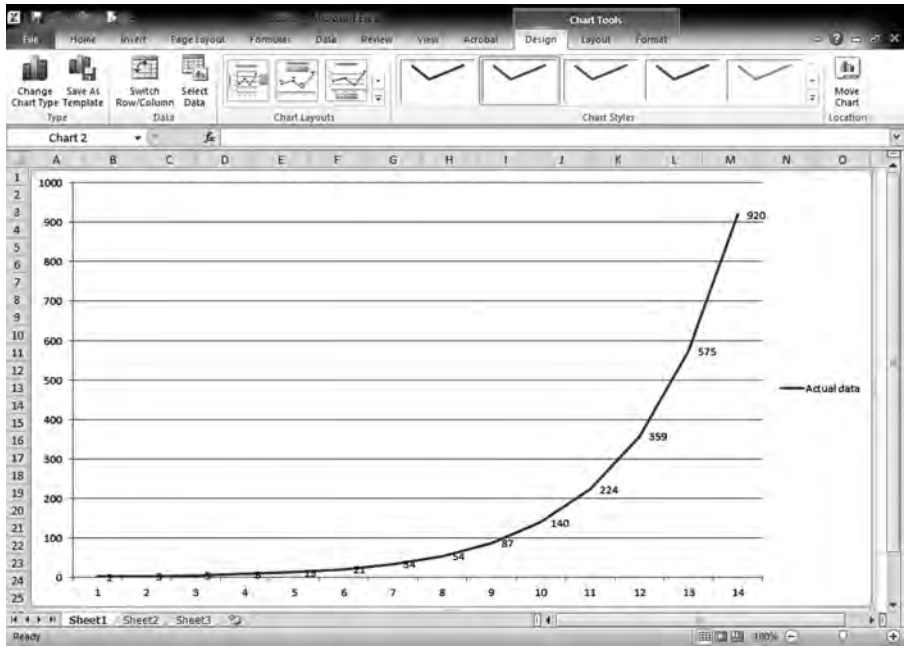

Fig. 3.1. Growth curve for data presented in Table 3.1.

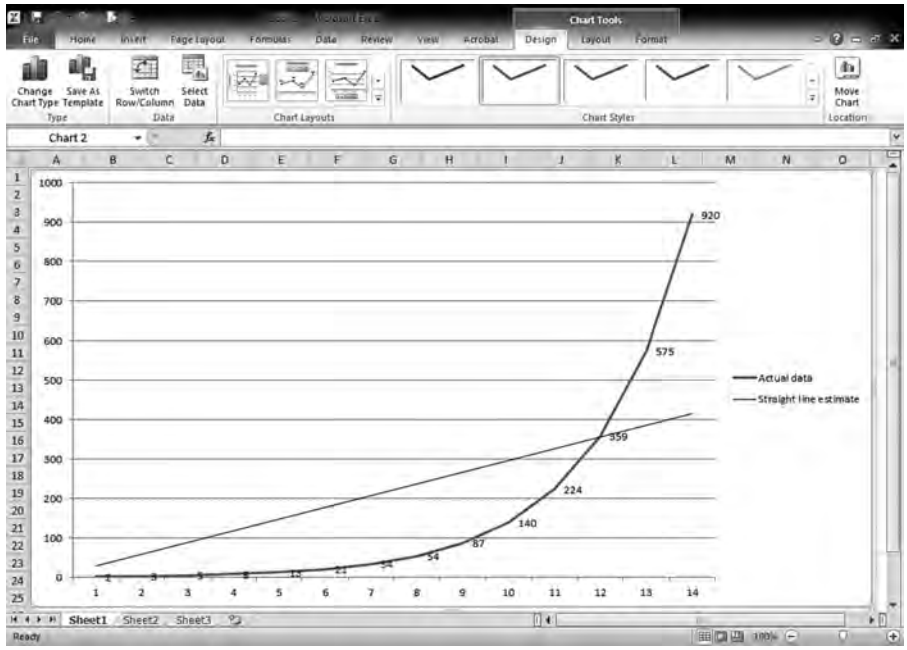

Fig. 3.2. A straight line 'fitting' the data presented in Table 3.1.

Table 3.2. Daily increase in epicotyl length for tomato seedlings measured for 14 days.

Day	Epicotyl length (mm)	Daily increase in length (mm)
1	2	0
2	3	1
3	5	2
4	8	3
5	13	5
6	21	8
7	34	13
8	54	20
9	87	33
10	140	53
11	224	84
12	359	135
13	575	216
14	920	345

Table 3.3. Daily increase in epicotyl length expressed as a percentage for the data presented in Table 3.1.

Day	Epicotyl length (mm)	Daily increase in length	
		(mm)	(%)
1	2	0	0
2	3	1	50
3	5	2	67
4	8	3	60
5	13	5	63
6	21	8	62
7	34	13	62
8	54	20	59
9	87	33	61
10	140	53	61
11	224	84	60
12	359	135	60
13	575	216	60
14	920	345	60

Growth is itself increasing over time. This type of curve often describes bacteria or yeast growth rates as well as the human population on earth.

The equation for an exponential growth curve is:

$y = ae^{bt}$

where t is time, and y is the growth variable, in our case, epicotyl length. The symbol 'e' is the base of natural logarithms and is equal to approximately 2.718.

The letters a and b are parameter symbols. Parameters are numbers that must be mathematically estimated. Since our equation is one of an

infinite number of exponential equations that may exist, the parameters *a* and *b* describe the one unique equation that fits our data.

These parameters can be estimated with many different software packages (or even by hand if you're so inclined). Microsoft Excel is a widely available program which is useful in plant growth modeling. Though there are other more sophisticated packages available, Excel is normally adequate for this purpose. In order to estimate the parameters *a* and *b* that uniquely describe the equation fitting our data, enter the data into a spreadsheet as shown in Fig. 3.3.

To estimate the parameters and fit the exponential curve to our data, first select 'Insert' and from the 'Charts' menu select 'Line', as shown in Fig. 3.4.

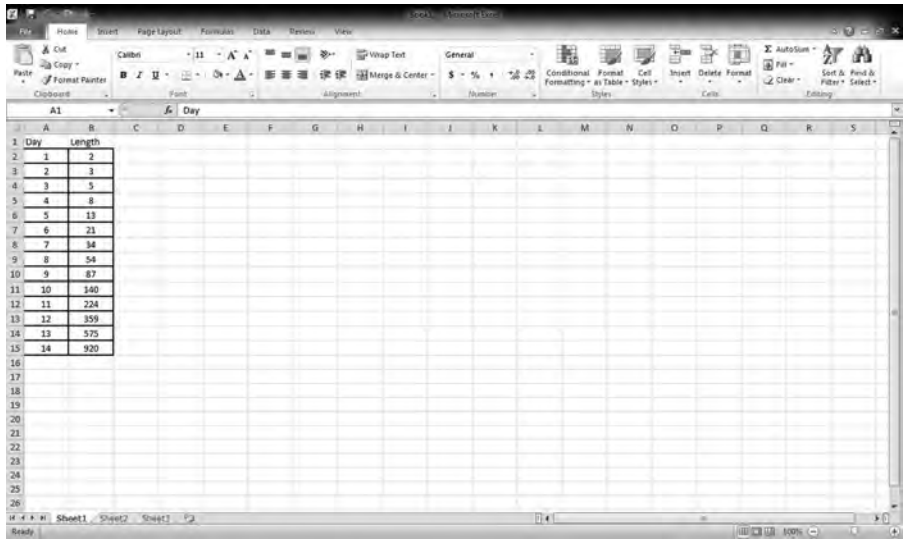

Fig. 3.3. The data for Table 3.1 entered into a simple Excel spreadsheet for estimating the parameters *a* and *b* of an exponential equation.

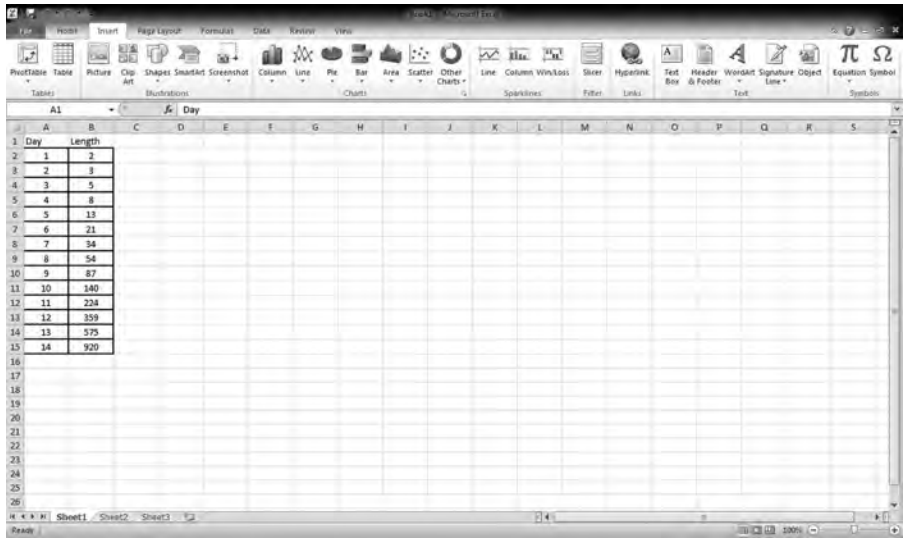

Fig. 3.4. Selecting 'Insert' and then 'Line' from the 'Charts' menu in Excel.

The chart should resemble Fig. 3.5.

The appropriate data must be selected (i.e. highlighted using the cursor) to produce the correct plot as shown in Fig. 3.6.

By right-clicking on the chart a menu appears from which click on 'Select Data' and this brings up a 'Select Data Source' pop-up window as shown in Fig. 3.7.

Change the chart data range to B2:B13. This will now produce a chart with only epicotyl length plotted against time as shown in Fig. 3.8. (We won't get into formatting the chart to look nice, we'll just see how to use Excel to get some quick information about our data.)

To fit an exponential curve to this data, right-click on the plotted line and select 'Add Trendline'.

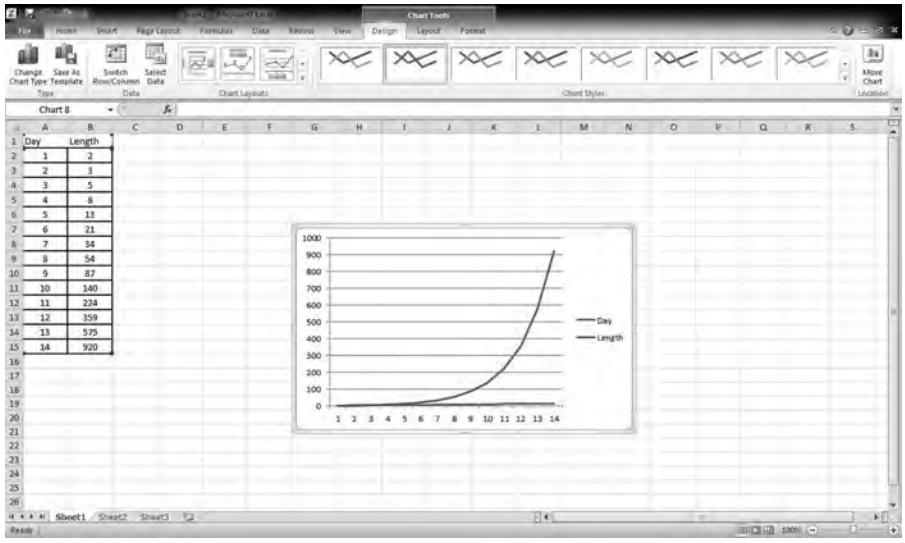

Fig. 3.5. A simple line chart for the Excel tomato epicotyl data.

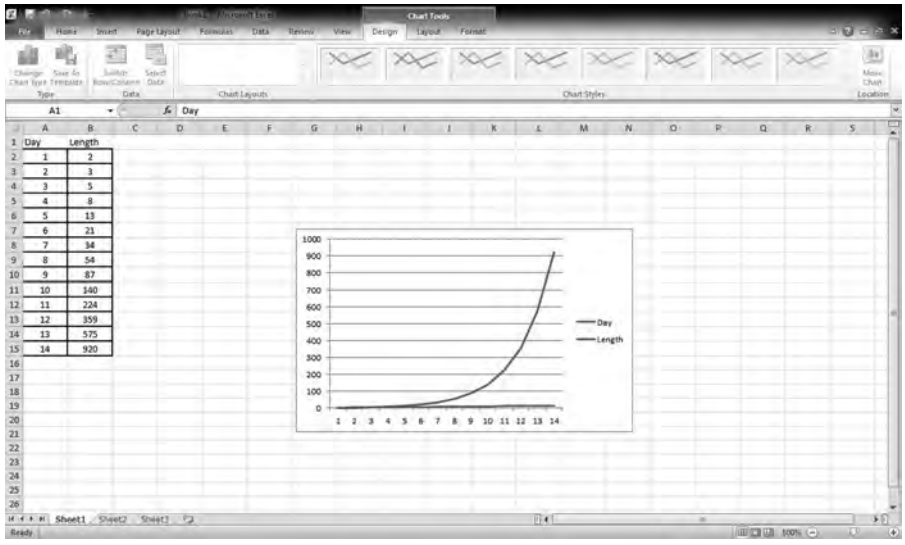

Fig. 3.6. Selecting the appropriate data to plot for the Excel tomato data.

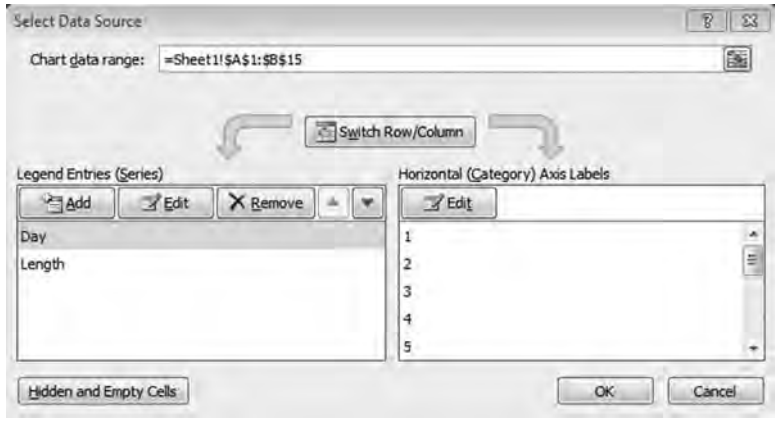

Fig. 3.7. 'Select Data Source' pop-up window for the tomato epicotyl data of Table 3.1.

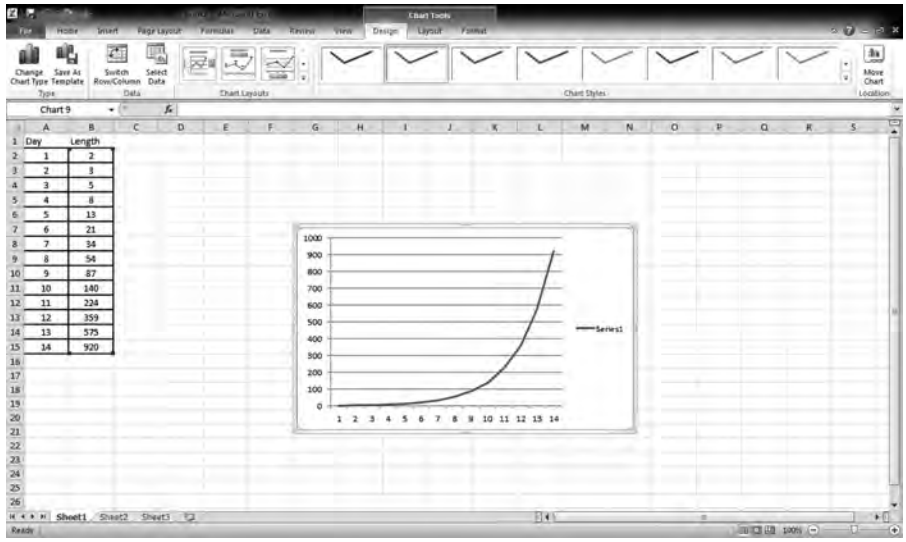

Fig. 3.8. Epicotyl length plotted against time for the tomato data of Table 3.1.

Select 'Exponential', 'Display Equation on chart' and 'Display *R*-squared value on chart' in the pop-up window as shown in Fig. 3.9.

This will cause Excel to provide the equation of the line and the *R*-squared (R^2) value for the equation on the chart (Fig. 3.10).

The equation describes the mathematical relationship between day and epicotyl length while the *R*-squared value gives an indication of how well the data fit the model chosen. *R*-squared values are between 0 and 1.00 with values closer to 1.00 considered a better fit.

From Fig. 3.10, we get the equation of our line as $y = 1.2092e^{0.4745x}$ with an *R*-squared value of 0.99 (a particularly good fit).

We can predict what the epicotyl length would be at any time between days 0 and 14 by plugging our x value of time into the equation. Don't go out farther than 14 days, since we did not make any measurements past day 14. This is called extrapolation and is not acceptable.

Normally to get a better estimate of the line we would measure more than one seedling and repeat the experiment several times. We would then alter

some factor during seedling growth, take measurements and determine if our alteration had any effect on the growth curve. We would use sophisticated statistical methods for comparing line equations to determine the effect of our alteration. Just for fun, ask Excel to fit a straight line and see what you get.

Fig. 3.9. The 'Format Trendline' pop-up window for selecting equation options in Excel.

Sigmoid

The sigmoidal growth curve consists of three (some scientists say five, but three will suffice for our purposes) main stages: (i) the lag phase; (ii) the log phase; and (iii) the stationary phase. The first stage, the lag phase, is characterized by an initial gradual increase in the growth parameter. This is followed by the second stage, the log phase, in which there is a rapid increase in the parameter. In the final phase, the stationary phase, there is a gradual decline in the rate of increase of the parameter. Usually the increase ceases and the curve levels out. If the organism is observed long enough, evidence of senescence might be observed by a gradual decrease in the growth parameter.

Fruit growth often follows the sigmoid growth curve. Remember the type of growth you might observe depends on the parameter you measure. For example, the number of cells in an organism may be increasing steadily while the organism's weight may not be changing much at all. In the first case you might say you are in a log stage of growth while in the second case, you may say you are in a lag stage of growth.

If we measure the weight of an apple as it grows on a tree, we can make some interesting observations which will help us understand the sigmoid growth curve. Consider the observations recorded in Table 3.4.

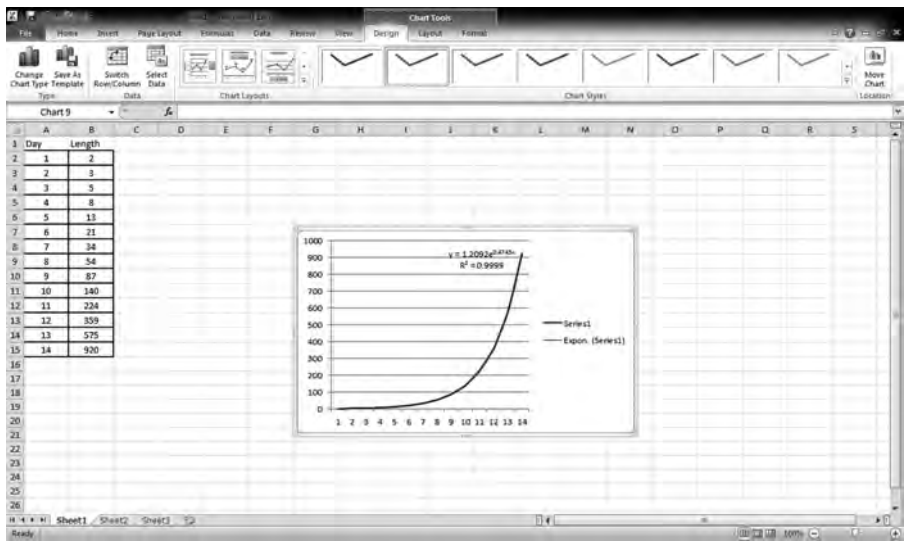

Fig. 3.10. The final Excel graph with equation and *R*-squared displayed.

Table 3.4. Fruit weight of an individual apple fruit at fertilization and for 24 weeks thereafter.

Weeks after fertilization	Fruit weight (g)	Weeks after fertilization	Fruit weight (g)	Weeks after fertilization	Fruit weight (g)
0	0	9	41	18	130
1	5	10	48	19	140
2	9	11	56	20	147
3	14	12	64	21	154
4	18	13	74	22	160
5	23	14	85	23	165
6	27	15	96	24	170
7	31	16	105		
8	36	17	118		

A plot of this data is presented in Fig. 3.11. Notice that fruit weight increases steadily up to around 9 weeks or so and then increased more rapidly until about 19 weeks where the increase in fruit weight gradually tapers off.

Mathematical modeling of a sigmoid, also called a logistic growth curve, is difficult. The Richards' growth function is often used when modeling a sigmoid relationship. For more information regarding mathematical modeling of plant growth, consult any good mathematical modeling text.

Double sigmoid

While an apple fruit follows a sigmoid growth curve, a peach fruit follows a double sigmoid growth curve. A double sigmoid curve can be thought of as two sigmoid growth curves joined together as what is called the d point. The first sigmoid curve represents growth of the fruit mesocarp (flesh), primarily by cell division and is often called phase I. The second sigmoid curve represents the period called final swell which is due to mesocarp cell enlargement, often called phase III. The d point is the period of endocarp or pit hardening, often called phase II.

What is Development?

Development versus differentiation

Differentiation and development describe a forward movement in plant growth. Differentiation usually refers to the specialization of cells followed by tissues for their ultimate function within the plant. Development usually refers to the forward growth of organs and the whole plant, all towards achieving the plant's ultimate horticultural functionality.

Although differentiation and development refer to forward growth and neither is reversible in the horticultural sense, de-differentiation can occur at the cellular level. Cells that once held a differentiated function may de-differentiate to form a mass of non-differentiated tissue called callus.

Development is part of differentiation. At the cellular level, differentiation is the process wherein a cell becomes the specific type of cell it was destined to become due to genetics and environment. All cells begin similarly. As their differentiation occurs, each takes on specific anatomical and physiological characteristics which fit their purpose. A collenchyma cell develops a thick, flexible cell wall while a root epidermal cell develops a root hair. Since all cells of a given organism have the same genetics, differentiation within an organism is largely directed by environment.

Development follows differentiation. Where you make the cut-off between the two is often subjective. In academic discussions, it is wise to explicitly convey your own definition of differentiation and development with respect to the plant material you are working with.

Growth of undifferentiated tissue into differentiated tissue

What makes a cell or tissue grow in one direction and not another? In other words, why does cell 'A' become a spongy mesophyll cell rather than a companion cell in a leaf? This is one of the mysteries of plant growth.

The differentiation of cells and tissues is highly regulated and not well understood. While we know how the many different factors influence the differentiation of plants on a macroscopic level, we have limited understanding of what is happening at a more basic level. For example, we may know that

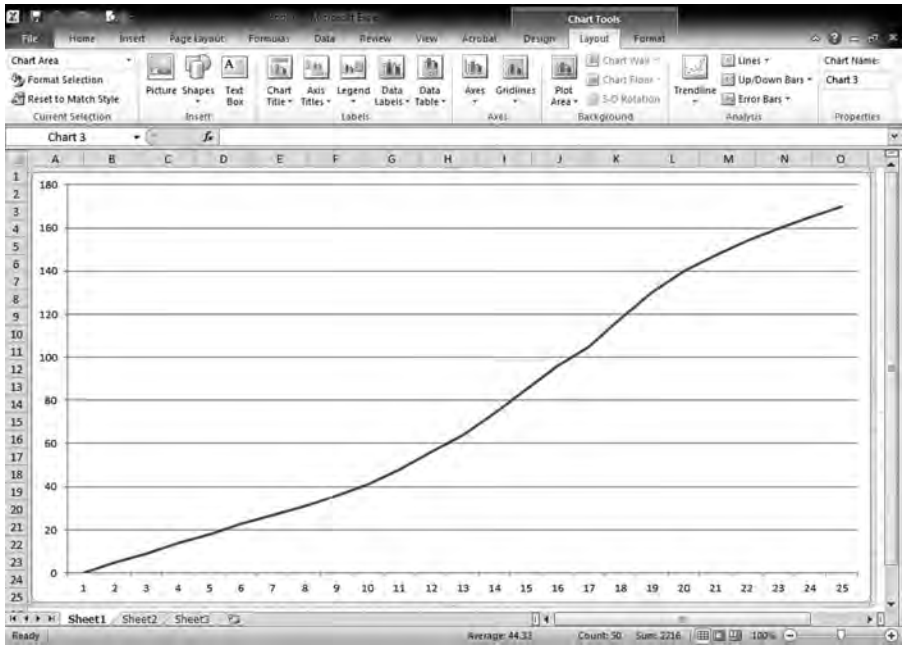

Fig. 3.11. A sigmoid growth curve of apple fruit growth from the data in Table 3.4.

short days may cause flowers to be initiated in certain species, but we don't really understand much of why the shorter days cause flowers instead of leaves to be formed by a meristem.

Development of differentiated tissue

Development is that part of the growth process where cells or tissues that are already differentiated continue to enlarge and attain their final form and function within the plant. The distinction between differentiation and development is that development can only proceed once all parts necessary for development exist. If all parts are not available for growth to progress, the cell or tissue is still in the differentiation process.

There may even be a lag between when differentiation ceases and development commences. We will see this clearly when we study flower formation. All parts might exist, but movement towards final form and function may not occur for some time.

Growth of plant from an embryo through maturity

A new plant that is formed via sexual reproduction (as opposed to asexual reproduction) starts out as a single-celled zygote formed from the union of an egg cell and a sperm cell. This single cell reproduces via mitosis to form a multi-cellular embryo. The embryo continues to increase in size via cell division and enlargement. Root tissues begin to become distinguishable from shoot tissues. Leaves begin to be distinguished from stems, and the development of the new plant proceeds. There may be a lull in growth as a seed lays dormant, waiting for conditions to be acceptable for further movement towards final form and function. Conversely, the embryo may not stall in its movement, and may quickly develop via germination into a young seedling. The seedling then proceeds towards its final destination.

Factors Affecting Both Growth and Development

The two main factors responsible for growth and development in plants are: (i) genetics; and (ii) the environment. Genetics hold the potential for what might happen in the course of plant growth while environmental factors control how the genetic potential is expressed.

Genetics is a simply elegant code for one of the most sophisticated processes on earth: life.

Essentially, four nucleotides (adenine, guanine, cytosine, and thymine) pair up (adenine with thymine and guanine with cytosine) to form sequences of base pairs which ultimately code for the 20 amino acids commonly found in proteins. The proteins formed from combinations of these amino acids form enzymes which run the whole show.

How do these sequences of base pairs run the show? In addition, how does the environment affect the expression of this life code? The first question is studied by geneticists while the second is studied by physiologists. Working together, lifetimes of research have been devoted to answering these questions.

In order for the expression of a plant's genetics to be influenced by the environment, a mechanism for the detection of environmental signals must exist. There are specific proteins in plants that change in response to environmental stimuli. One of the most highly studied signal-catching proteins in plants is phytochrome, and the signal it catches is light. This molecule changes shape when exposed to light of differing wavelengths, particularly red (660 nm) and far-red light (730 nm). The phytochrome molecule's shape or form, most often called P_r or P_{fr}, then influences what sort of physiological response occurs. But how?

The idea is that the original light signal perceived by the plant (or other signals in other situations) is passed along within the plant itself via secondary messages in a process called transduction. This secondary message may directly affect some process or it may induce a change in or synthesis of another protein (remember enzymes run the show) which may lead to the actual response. Remember, all life processes are run by enzymes and all enzymes are proteins.

Generally the result of signal transduction is in the activity of an enzyme. The enzyme activity can be regulated in either of two ways. The first is called 'transcriptional regulation'. Here factors such as modified proteins or chemical messengers, resulting from the modification of a protein, directly bind to specific regions of DNA and control the transcription (the conversion of the code contained in DNA to RNA) of specific genes which code for the specific enzymes important in some physiological process. In other words, the synthesis of an enzyme is controlled by the signal. The second way is when the activity of an already-existing enzyme is regulated by the signal. This is called 'post-translational modification of proteins'.

In summary, whatever the environmental signal is (light, temperature, gravity, carbon dioxide concentration, etc.), a plant generally perceives the signal via a protein molecule. The protein molecule then sends the signal along a chain of events which ultimately results in some sort of physiological response by the plant.

Plant Movements

One of the areas where plant signal perception is highly noticed is in plant movements (Fig. 3.12).

Autonomous

Some plant movements are autonomous and are not caused directly by some external stimulus. The most commonly observed autonomous movement is circumnutation. This is a slow, circular movement of shoot or root tips or tendrils as they grow. The movement is most easily observed using time-lapse photography as each complete circle takes from 1 to 3 h to complete. This movement is caused by differential growth of cells in the growing tissue. Another example of autonomous movement is when a stem wilts and bends due to desiccation. The stem wilts due to a physical change in the turgidity of the cell, not due to some external stimulus.

Induced

Induced movements are those plant movements directly caused by some external stimulus. There are two types of induced movements: (i) nastic; and (ii) tactic.

Nastic movements

Nastic movements are plant movements that are independent of the direction of the stimulus. The most widely cited example is seismonasty which is a special case of thigmonasty which is characterized by an especially fast reaction to a stimulus by a plant. Thigmonasty is a response due to touch. When a *Mimosa* leaf is touched the leaflets fold upwards and that folding is not oriented in any direction related to the direction of the touch. You can touch the top, bottom or sides of the leaf and the leaflets will always fold up.

In addition you can stimulate the leaflets to fold (again always upwards) by heating or cooling the leaf.

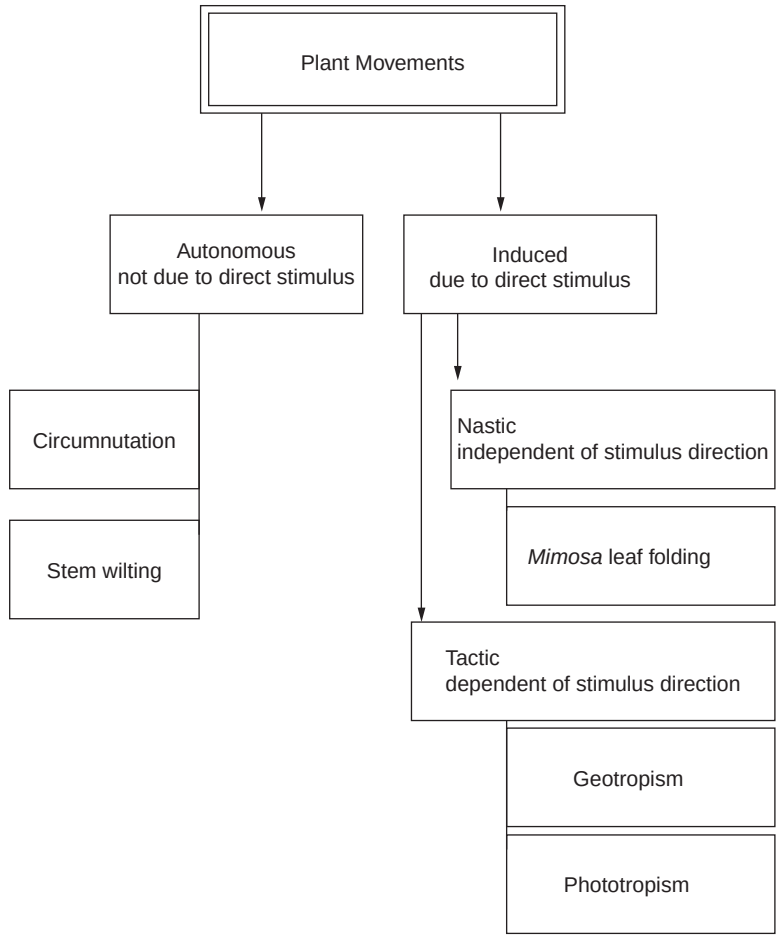

Fig. 3.12. The different types of plant movements.

But how do the leaflets fold up from a physiological standpoint? The stimulus (mechanical, heat, cold) causes an electrical signal to move from cell to cell down the leaf very rapidly. When the signal reaches specialized cells called 'motor cells' in a structure called the pulvinis at the base of the leaflet, a rapid efflux of potassium ions out of the motor cells occurs. Water molecules quickly follow, the motor cells loose turgor and the leaflets fold upwards. Once the stimulus is gone, potassium slowly re-enters the motor cells, water follows and turgidity is restored causing the leaflets to re-open. The potassium ion movement and hence leaflet folding can also be controlled photoperiodically.

Another example of thigmotropism is tendril curling around a support.

Nastic movements are typical in dorsiventral organs such as leaves. Dorsiventral describes an organ that has two sides, each differing in structure and appearance. Tissues within a dorsiventral organ have different growth capacities on the upper versus the lower surface. Uneven growth of the upper and lower surfaces results in a predetermined direction of movement. Regardless of the direction of the stimulus, the resultant movement is always in the same direction.

Other nastic movements include the opening and closing of flowers due to some external stimulus. Flowers of members of the family *Cucurbitaceae* open during the day and close at night. This regulation by light is called photonasty. Tulips (*Tulipa* spp.) and crocus (*Crocus* spp.) flowers open when warm

and close when cool (thermonasty). Epinasty is the downward curving of leaves induced by some external stimulus such as ethylene gas. Chemotropism is movement induced by a chemical substance. For example, digestive glands of the insectivorous plant *Drosera* curl inwards as a response to the nitrogen in their insect prey.

Tactic movements

Tactic movements are often observed in organs of radial symmetry like a shoot or root and are oriented in either the same or opposite direction of the stimulus causing the movement. Two widely observed examples are phototropism and gravitropism (also called geotropism). In phototropism, movement is towards the stimulus (i.e. a plant bending towards a light source). In gravitropism, the direction of the movement depends on the organ. Movement is away from gravity when discussing the shoot, while it is towards gravity when discussing the root. A clear example of gravitropism can be observed by placing a potted plant on its side and watching it curve to grow upright again. The response can be observed within 15 min!

Turgor versus growth movements

Many plant movements are due to either local growth responses to a stimulus or changes in turgor of selected cells within tissues responsible for the movement. These responses rely on the elasticity and plasticity of cell walls.

Plasticity of the cell wall is the result of the deposition of new cell wall material into a stretched or stretching cell wall in order to stabilize it and results in an irreversible increase in cell volume. An example would be a cell on the non-illuminated side of a stem which stretches with the resultant bending of the shoot towards a light source.

Elasticity is the ability of the cell wall to stretch without the deposition of new wall material which results in a reversible change in cell size. An example of is the motor cell responsible for *Mimosa* leaflet folding. As water moves into the cell in response to the influx of potassium ions, the cell walls stretch and the cell enlarges. When potassium ions leave the cell in response to a stimulus, water also leaves resulting in a decrease in cell size.

The movements of *Mimosa* leaflets are based on reversible turgor changes and are often called turgor movements to distinguish them from growth movements. Bending of a shoot towards the light or away from gravity is a growth movement and is not reversible.

In turgor movements, changes in pressure in one cell may exert pressure on neighboring cells which may lead to tissue deformations and plant movement. If the cells are different sizes, the pressure is transmitted in very specific directions causing very specific movements. Other examples of turgor movements include the opening and closing of stomata and the circadian lifting and falling of leaves, such as that seen in *Phaseolus* sp.

In growth movements turgor pressure increases as a result of a stimulus and does not decline afterwards. This results in an irreversible growth movement. Sometimes the pressure continues to build causing tissue rupture. Seed pod rupture in *Impatiens* is a good example of this type of irreversible movement.

Stimulus perception, forwarding, and conversion into movement

A series of metabolic processes which are coded for by the genetics of the organism are responsible for autonomous movements. There is no identifiable stimulus responsible for the movement.

Induced movements, however, require a signal, its perception, forwarding to the appropriate tissue, and conversion into the actual movement.

A model of signal perception is fairly straightforward using light perception as an example. The light signal is perceived by the plant via specialized molecules called pigments. When light of a specific wavelength hits the molecule, the energy in the light is used to change the structure of the molecule. The pigments responsible for light signal perception in plants include blue light receptors called phototropins and cryptochromes, and the red light receptor phytochrome.

Let's look at phytochrome. Only specific wavelengths of light will convert one form of the molecule to another. Red light will convert phytochrome red (P_r) to phytochrome far red (P_{fr}) and only far-red light will convert P_{fr} back to P_r. P_{fr} stimulates shoot elongation, which for this discussion we will consider a plant movement (even though some would argue that shoot elongation really isn't movement per se, but rather simply irreversible growth). Once enough red light has caused enough accumulation of P_{fr}, how is the light signal forwarded to cells to cause them to elongate?

The forwarding of the perceived signal and its ultimate conversion into movement is not understood as well as signal perception.

The coleoptile, a tubular protective sheath surrounding the young shoot of a germinating grass seedling, is quite responsive to phototropic bending. There is work which suggests that the actual site of light signal perception in oat (*Avena*) coleoptiles is the chloroplast. Coleoptiles grown in the dark are etiolated and accumulate violaxanthin and antheraxanthin but no zeaxanthin. All three compounds are yellow xanthophyll pigments in the carotenoid group of plant pigments.

If dark-grown coleoptiles are irradiated with red light, zeaxanthin accumulates in proportion to the length of time they are irradiated. Thus phytochrome is important in regulating zeaxanthin synthesis, since irradiating with red light would cause a conversion of P_r to P_{fr}, the active form of phytochrome.

If coleoptiles with differing levels of zeaxanthin are exposed to brief bursts of blue light, their phototropic bending is proportional to the amount of zeaxanthin present. If there is no zeaxanthin present, there is no phototropic response. Thus the coleoptile bending is regulated by both blue and red light. The red light regulates the production of zeaxanthin and the blue light regulates the bending by acting on the zeaxanthin. (Guard cell movement (stomatal opening and closing) is also sensitive to blue light and depends on the presence of zeaxanthin in the guard cell chloroplasts.)

But how does the light regulate the levels of the pigments controlling the tropic response? Different colors of light affect different pigments (phytochrome, phototropins, and cryptochromes) which probably promote the formation or activity of enzymes responsible for the synthesis of other pigments (zeaxanthin) which pass the signals message onto other enzymes.

What other enzymes? Well we know that one of the plant hormones, auxin, is synthesized in coleoptile tips when irradiated with light. We also know that auxin diffuses basipetally down the coleoptile where, when it reaches the sub-apical cells of the coleoptile, it accumulates on the dark side of the shoot. Even though light stimulates auxin production, it also causes auxin degradation. We also know that auxin makes cell walls more elastic, thus allowing turgor pressure to enlarge the cells on the dark side of the shoot. The cells on the light side of the shoot do not elongate (no auxin to loosen the cell wall), thus there is an uneven growth of the stem with the dark side longer than the light side. The shoot bends toward the light!

4 Physiology of Growth in Specific Organs: Roots, Stems, and Leaves

In this chapter we will investigate the physiology of some specific growth characteristics of roots, stems and leaves to get a handle on how plants are able to grow and prosper as stationary organisms in an incredibly dynamic environment. Other organisms have the luxury of being mobile which allows them means of avoiding certain obstacles to their growth and development. Plants on the other hand are stuck where they are and must utilize some pretty fascinating mechanisms to cope. Some of the mechanisms we'll look at are stress avoidance mechanisms while others are simply growth responses to a myriad of environmental signals.

Roots

Geotropism (gravitropism)

Roots grow down because of gravitropism, a tactic plant movement in response to the pull of gravity. Remember that tactic means the response is related to the direction of the stimulus. Roots move towards the gravity stimulus, thus the gravitropic response of roots is considered a positive tactic response. Shoots move away from the gravity stimulus, thus the gravitropic response in stems is considered a negative tactic response. In the context of plant physiology, positive and negative have nothing to do with good or bad, but rather is referring to the direction of the response. In tissues that grow seemingly neutral to the stimulus, such as leaves or sideways growing roots, the response is considered plagiotropic.

If a seedling is placed on its side, the roots will begin to grow downwards and the shoot will begin to grow upwards, both gravitropic responses occurring at the same time in the same plant. The upright growth of the shoot could be at least partially attributed to light, but this response to light is really secondary since shoots will grow upright, away from gravity even in the dark.

The starch-statolith hypothesis

The most widely accepted hypothesis of how plants respond to gravity is called the starch-statolith hypothesis (Fig. 4.1). This hypothesis has been around for over 100 years and continues to gain acceptance today (Blancaflor and Masson, 2003; Perrin *et al*., 2005).

SIGNAL PERCEPTION Cells which detect the gravitropic signal are called statocytes. The statocytes in roots are specialized cells called columella cells. They are located just behind the root cap itself. If columella cells are carefully examined, small grains of starch called statoliths can be seen in plastids known as amyloplasts. The statoliths, being rather dense and heavy, will settle to the bottom side of the columella cell. Whenever a root's orientation is altered, the statoliths will settle to the side of the cell closest to the gravity stimulus. Thus the mechanism for signal perception is the location of statoliths in columella cells (Chen *et al*., 1999). But the columella cells do not directly respond to the stimulus (i.e. their growth does not change). It is the cells in the growth zone (also called the zone of elongation) which change. There has to be some signal transmitted from the cells containing the statoliths to the cells in the growth zone that causes the growth of the growth-zone cells to change.

In shoot gravitropism, the statocytes are endodermis or bundle sheath cells. In these cells, statoliths sense a change in the direction of the pull of gravity in much the same way as in root tissue. The position of statoliths in these cells generates a signal that moves laterally to peripheral tissues causing differential growth there which results in shoot bending. Again, the site of signal perception is not the same as the site of the response to the signal, thus some sort of signal transmission must occur.

CELLULAR PERCEPTION The perception of gravity in plant cells relies on statolith position. But how is their position detected? There are filaments

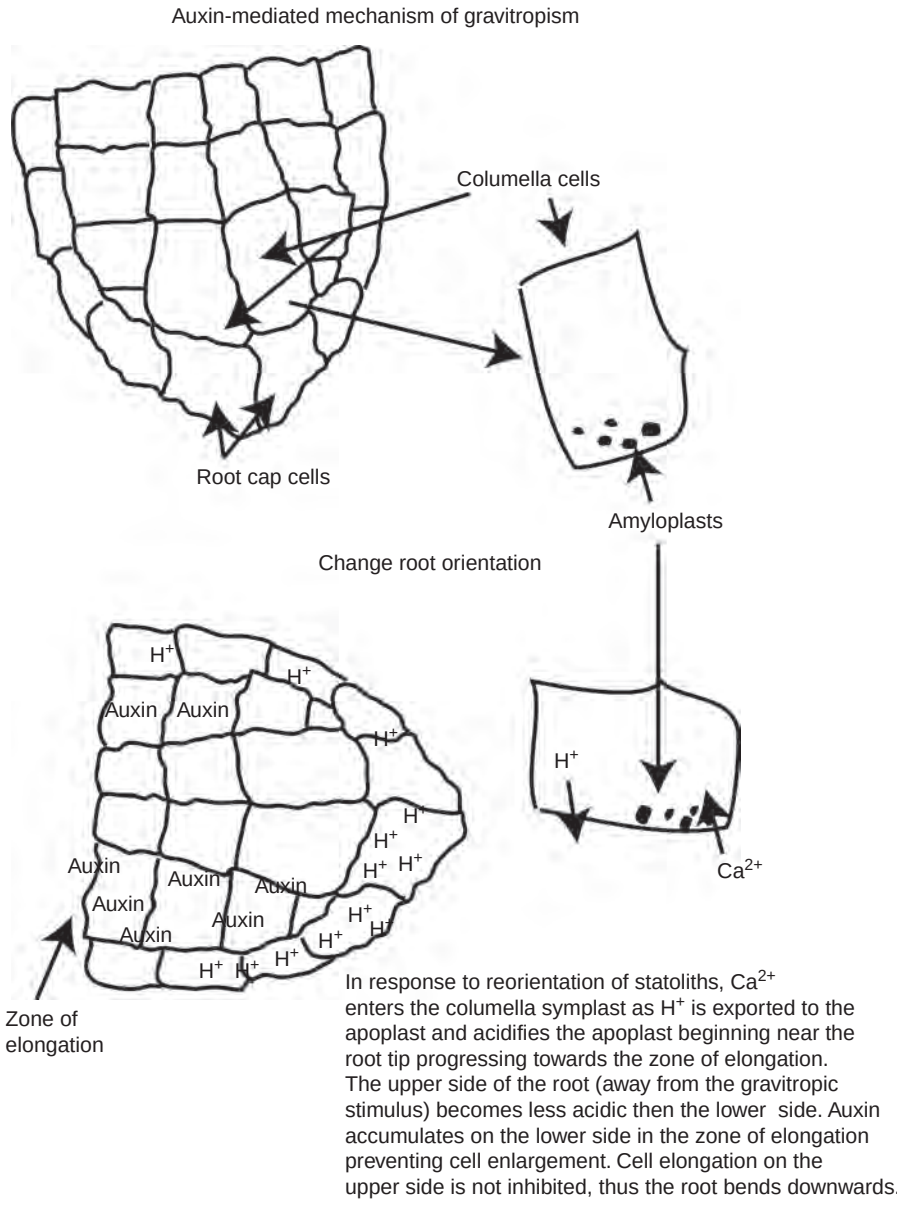

Fig. 4.1. The starch-statolith hypothesis of geotropism.

of proteins throughout the cellular cytoplasm. The thin filaments are composed of the protein actin, while thicker, tubular filaments called microtubules are composed of the protein tubulin. These filaments and microtubules are attached to many cellular components and are important in many cellular processes including cell division, cell signaling, cell expansion, and overall cell structure.

Statoliths travel through this network of filaments as they move in response to gravity. This disruption of the network causes a response at the plasma membrane. The response is due to a mechanical disruption of the filaments by the statoliths which causes a change in the tension exerted by the filaments at their points of attachment to the membrane. The change in tension at the membrane

triggers the movement of calcium ions into the cytoplasm through specific protein channels in the membrane with a concomitant movement of protons out of the cell (Yoder *et al*., 2001; Boonsirichai *et al*., 2002).

The protons moving out of the cell acidify the apoplast (the area outside of and between cell membranes). An asymmetric change in root surface pH is first observed at the root cap and then progresses along the root to the elongation zone. The side of the root closest to the gravity signal becomes more acidic than the side opposite the signal. The acidification of the root surface seems to influence the activity and/or distribution of auxin in stimulated root tissues (Scott and Allen, 1999; Fasano *et al*., 2001).

AUXIN PRODUCTION, TRANSPORT, AND GRAVITROPIC CELL GROWTH Auxins are mainly synthesized in young shoot tissue, and are transported passively through the phloem into the root. In general, auxin inhibits elongation in root cells and promotes elongation in shoot cells. Auxin movement is called polar transport, indicating that movement is due to a gradient from regions of high concentration to regions of low concentration. This movement is accomplished by means of transmembrane transporters, proteins specifically designed to move auxin. One type of transporter moves auxin into a cell and another moves auxin out. The auxin transporters may be distributed asymmetrically in cells which can lead to asymmetric auxin concentrations in cells, depending upon the particular circumstance. This model of auxin transport is called the fountain model (Wolverton *et al*., 2002).

ARABIDOPSIS Much of our understanding of gravitropism comes from work with *Arabidopsis thaliana* (L.) Heynh. *Arabidopsis*, also known as mouse-ear cress, is a small flowering plant in the family *Brassicaceae* (mustard family) which has little horticultural significance as a crop, but is extremely important in the study of basic molecular mechanisms in flowering plants. Its small genome with only five chromosomes has been completely mapped. It produces an abundance of seed in only 6 weeks from germination, thus the turn-around time for genetic studies is very short. Since a mature plant is only 20–25 mm tall, it takes up very little space when cultivated. Additionally, many mutant lines have been identified and genetic transformation is fairly easy using *Agrobacterium tumefaciens*.

***ARABIDOPSIS* AND THE AUXIN STORY** Auxin produced in young shoot tissue is transported through the phloem to the root tip from cell to cell via auxin transporters. The genes responsible for coding the proteins responsible for transporting auxins have been extensively studied in *Arabidopsis* (Friml, 2003). The transporters responsible for auxin influx are coded for genes collectively called AUX while those responsible for cellular efflux of auxin are coded for by genes collectively called PIN. Auxin must be transported from the vascular tissue into the root tip through protophloem cells. The AUX transporters are located primarily on the basal side of the membrane while the PIN transporters are located on the apical side. Remember in the case of root cells, basal is up and apical is down. Thus the movement of auxin will be in an acropetal direction, away from the vascular tissue to the root cap.

At the root cap, a specific transporter (PIN4) causes auxin to accumulate in the upper layers of columella cells in the root cap. In these cells, a specific transporter called AUX1 ensures uptake by these cells and a specific transporter called PIN3 regulates efflux. Where PIN3 is located on the membrane depends on root orientation. When the root is oriented vertically, PIN3 is symmetric on the plasma membrane of columella cells. When the root is reoriented, PIN3 relocates to a position now sensed to be the new cell bottom. The distribution of PIN3 in the root columella cells could be related to changes in the filament network brought about by statolith settling or by gravity-induced changes in calcium and pH caused by statolith reorientation.

With the PIN3 efflux transporters located at the bottom of the cell, there is an efflux of auxin into the apoplast on the lower side of the root with a concomitant increase in apoplast auxin concentration. The auxin then moves basipetally towards the elongation zone via passive polar transport. The increased auxin concentration in elongation-zone cells reduces their elongation growth (remember, auxin inhibits cell elongation in root tissue). On the opposite side of the root, auxin concentrations have not increased, thus elongation-zone cell elongation is not inhibited. Since cell elongation on the lower side of the root is inhibited while elongation on the upper side of the root is not, the root curves downwards.

When a shoot is tipped on its side, statoliths collect on the bottom side of the stem resulting in an

efflux of auxin from cells, which is probably due to the reorientation of PIN3 transporters. Auxin would then accumulate on the lower side of the stem, promoting cell elongation (remember auxin promotes cell elongation in shoot tissue). The shoot would then curve upwards due to the differential cell elongation caused by auxin.

Cytokinin gradients may also play a role in the root gravitropic response, however, evidence is still limited for this involvement (Wolverton *et al*., 2002).

BUT HOW CAN AUXIN STIMULATE CELL ELONGATION IN SHOOTS YET INHIBIT IT IN ROOTS? This phenomenon is likely to be due to differences in cell sensitivity to auxin concentration. Root cells are much more sensitive to auxin than stem cells. Low concentrations elicit a response in root cells but have no effect on stem cells. Levels needed for a response in stem cells are inhibitory in root cells.

For example, let's say that the optimum auxin concentration needed for root cell elongation is 10 ppm. Stem cells do not elongate with 10 ppm auxin since they are less sensitive to auxin concentration than root cells. Increasing the auxin level to that needed for stem cell elongation raises it to a level that is inhibitory to root cell elongation. The amount of auxin released by the gravitropic response must be near the optimum level for stem cell elongation, but is at an inhibitory level for root cell elongation.

This type of growth response is quite typical in plants. Think about light levels, water levels, and fertility. Most plants have a level of each that is optimal for growth, above or below these levels, growth is inhibited. It's no different on the cellular level. Why root and stem cells are different in their respective sensitivities to auxin levels is still a mystery.

The gravitational pressure hypothesis

Work with starchless mutants of *Arabidopsis* has led to an alternate hypothesis that the response to gravity is due to the perception of the weight of the entire cell, not starch granules (Wolverton *et al*., 2002). In general, the gravitational pressure hypothesis for responses to gravity seems only to be applicable to single-cell algae such as *Chara* spp. or unicellular flagellates such as *Euglena gracilis*. The possibility that an alternate gravity sensing mechanism exists in higher plants has been supported in work with corn (*Zea mays*) roots. Root growth continued to curve as long as the elongation zone was held at any angle away from vertical even when the root cap was vertically aligned. This observation suggests that the cells in the elongation zone could perceive the direction of gravity, even though they themselves do not contain amyloplasts.

Thigmotropic responses in roots

Roots naturally have a propensity to grow downwards into the soil due to gravitropism. When a root encounters an obstacle as it grows, it will turn and grow around the obstacle, apparently overriding the gravitropic response. When small flat pieces of material are place next to vertically growing roots, the roots will exhibit a thigmotropic response by bending away from the objects point of contact.

In laboratory experiments with *Arabidopsis*, roots will compromise their gravitropic response when they come in contact with a glass plate and exhibit a thigmotropic response instead (Massa and Gilroy, 2003). It is not surprising then that thigmotropic stimuli can delay the onset of a gravitropic response at the cellular level. Touching a root apex can delay one of the first-known gravitropic responses, that is the settling of starch granules in columella cells.

Stems

Phototropism

The physiology of plant stem growth includes a wide variety of stem growth responses to environmental stimuli. Since stem response to light in the form of phototropism is such an extensive subject, it is covered on its own in Chapter 8, this volume.

Thigmotropism

Plant response to any form of touch stimulus is called a thigmo response (Jaffe *et al*., 2002). Thigmotropic responses are those responses that occur directionally, based on the direction of the stimulus (e.g. the movement of a tendril winding around a trellis). Thigmonastic responses occur independently of the direction of the stimulus, such as the closing of *Mimosa pudica* leaves (Braam, 2005).

Tendril movements, vine coiling, and holdfast attachment

As part of their growth and development, many plants have the ability to climb via stem coiling, tendril grasping, or holdfast attachment. Each mechanism is an intriguing physiological activity developed by certain species over millennia of evolution. Part of the climbing response might be considered phototropic as the plant is moving towards a light source while another component may seem gravitropic as the stem is growing away from gravity. The responses are actually more specific thigmotropic responses at the tissue and cellular level.

Tendril movement

Some species have specialized appendages called tendrils specifically adept at coiling around solid objects which facilitates better exposure to sunlight for photosynthesis. These thin, long, often threadlike organs have a high degree of sensitivity to touch and friction on solid bodies which allows them to wind tightly around stakes, string, or even shoots, giving the appearance that they are physically climbing their support (Jaffe and Galston, 1968).

Plant families with tendrils are relatively few especially when compared with the number of families that have developed other mechanisms for climbing such as stem twining.

TENDRILS FROM STEMS Tendrils can originate from various plant organs. Some tendrils are modified shoots, such as those found in grapes (*Vitis* spp.) (Fig. 4.2), passionflower (*Passiflora* spp.), porcelain berry vine (*Ampelopsis glandulosa*), and evergreen grape vine (*Rhoicissus capensis*). Shoot tendrils originate as a shoot apical meristem and may have minute leaf primordia.

TENDRILS FROM LEAVES Some tendrils originate from a node as a specialized leaf where the blade never really forms and the central axis elongates via apical and intercalary growth. Examples of plants with so called 'leaf tendrils' include garden peas (*Pisum sativum*), sweet peas (*Lathyrus* spp.), many members of the family *Cucurbitaceae* (Fig. 4.3), cup and saucer vine (*Cobaea scandens*), and the Chilean glory flower (*Eccremocarpus scaber*). Leaf tendrils are often further categorized based on their specific origination within the leaf tissue complex.

Fig. 4.2. Grape (*Vitis* spp.) tendril which originates as stem tissue.

TENDRILS FROM LEAF TIPS Some species form tendrils from the tips of developing leaves. *Mutisia* spp., a South American flowering vine, can have leaf-tip tendrils. Asian pitcher plants (*Nepenthes*) form a tendril on the leaf tip. The tip of this tendril then develops into the pitcher.

TENDRILS FROM LEAFLETS A tendril can also form from a leaflet of a compound leaf (*Vicia, Lathyrus, Pisum*). With this type of tendril, the leaflet tendril, a single leaflet may develop into a tendril (as in many members of the family *Bignoniaceae*) or several of the most distal leaflets may become tendrils (as in *Lathyrus* spp.). Clematis is a temperate zone climber (family *Ranunculaceae*) with leaflet tendrils. *Cobaea*, a vine of the phlox family (family *Polemoniaceae*), forms tendrils from distal leaflets.

Fig. 4.3. Winter squash (*Cucurbita* spp.) tendril which originates as leaf tissue.

TENDRILS FROM STIPULES Stipules are small appendages at the base of a leaf. Greenbriar (also known as catbriar) (*Smilax*) has tendrils formed from stipules.

TENDRILS FROM LEAF STALKS Clematis (*Clematis* spp.) forms tendrils from petioles (leaf stalks).

TENDRILS FROM FLOWERS Pedicel or peduncle tendrils develop from the axis of an inflorescence. Chewstick (*Gouania lupuloides*), a tropical plant, forms tendrils from peduncles. Certain snapdragons (*Antirrhinum* spp.) have tendrils that appear to be derived from pedicels in the inflorescence.

TENDRILS FROM ROOTS Tendrils of the tropical orchids we use as flavoring (*Vanilla* spp.) originate from roots.

Tendril stimulation and direction of movement

Even though tendrils may originate from various plant organs, their movement and growth will be covered in this section on stem thigmo responses since the mechanism for such is the same regardless of origin.

Tendril movement can be either thigmonastic or thigmotropic depending on whether the direction of stimulation has an effect on the direction of coiling. If it does not, the response is thigmonastic, if it does, the response is thigmotropic (Jaffe and Galston, 1968). Some tendrils can bend in any direction when stimulated on any side. Others can only be stimulated and only bend on the ventral (lower) side, while others can be stimulated on all sides but only bend ventrally. Regardless of the type of bending possible, there are two stages of movement in the bending process.

The first stage is an autonomous movement which does not rely on any particular stimulation. Before a tendril begins to elongate and grow, it is often coiled. It uncoils as it begins to grow and its tip exhibits a circling movement which is caused by increased growth on the upper side of the tendril. By itself, this type of growth would lead to a spiraled tendril. The spiral is prevented by a migration of the growth zone in a screw-like fashion along the longitudinal axis. If a tendril reaches its full length as determined by genetics and has not found any support to coil around, it will wither and die or recoil.

The second stage of movement is an induced movement which begins when the tendril encounters a support. This could be a pole, a string, a stem, or even another tendril. When the support is encountered, a directed growth begins to occur. The tip of the tendril begins to wind itself tightly around the support.

Some of the cells on the epidermis of the tendril are called irritable cells. There are openings called touching pits in the cell wall of these cells which allow for the direct contact between the plasma membrane and the support. Upon contact, a signal is sent such that the turgor of ventral cells (cells closest to the support) decreases while turgor of dorsal cells increases. Dorsal cells therefore elongate more than ventral cells, causing the tendril to coil around the support. The change in turgor is probably due to changes in membrane permeability and flow of ions using metabolic energy.

The distal portion of a tendril is quite selective in its sensitivity. Stimulation as light as a 0.25 mg thread can evoke a response (coiling) in a matter of seconds, yet raindrops touching the tendril elicit no response (Simons, 1992). In addition, the stimulation causing coiling must remain present to maintain coiling, at least until cell wall lignification occurs. Transient stimulation may result in coiling, but the response is often reversed by uncoiling.

In some species basal coiling may occur after the initial tendril tip coiling. This type of basal coiling is called free coiling (Jaffe and Galston, 1968). Its purpose seems to be to bring the stem closer to the support that initiated the coiling at the tip. This suggests that the signal at the tip is

propagated down the tendril to the base where physiological changes induce basal coiling.

Many substances have been investigated as possible chemical messengers for inducing coiling. One such substance, 12-oxo-phytodienoic acid (ODPA), can induce coiling without mechanical stimulation in some species (*Bryonia dioica*, a relative of the cucumber) but not others (*P. sativum*, the garden pea). Application of ODPA leads to the accumulation of indole-acetic acid (IAA), which itself stimulates coiling (Weiler *et al*., 1993, 1994; Blechert *et al*., 1999). Even though ethylene increases with the application of either ODPA or IAA, it does not directly induce coiling since if ethylene synthesis is chemically blocked, coiling still occurs with ODPA or IAA (Weiler *et al*., 1993).

Other mechanisms of climbing

ADHESIVE PADS Some species such as Boston ivy (*Parthenosissus tricuspidata*) and Virginia creeper (*Parthenosissus quinquefolia*) climb via stem tendrils that have touch-sensitive adhesive pads. In these vines, tendril coiling is not a prerequisite for climbing as the adhesive pads will stick to almost any surface.

SHOOT TWINING There are many plants which do not form tendrils, yet still climb to great heights. One of the mechanisms responsible for climbing is shoot twining. Shoot twining consists of the encircling growth of a shoot around an object as the shoot elongates. Some common plants which exhibit shoot twining include morning glory (*Ipomea* spp.), green beans (*Phaseolus vulgaris*) (Fig. 4.4), clematis (*Clematis* spp.), and honeysuckle (*Lonicera* spp.). There are two different types of twining shoots: (i) twining via stems; and (ii) twining via leaves.

Plants which twine via leaves include clematis, climbing nasturtium (*Tropaeolum polyphyllum*), and *Rhodochiton*. In these plants, young leaves are able to twine around slender objects as the leaves elongate. In doing so the entire shoot is supported.

Many plants have stems which are able to twine around objects. The direction of twining (clockwise or counterclockwise) depends on the species. Some species that exhibit stem twining include green beans (*P. vulgaris*), black-eyed Susan (*Thunbergia*),

Fig. 4.4. An example of stem climbing by twining in green bean (*Phaseolus vulgaris*).

Dutchman's pipe (*Aristolochia*), bittersweet (*Celastrus scandens*), morning glory (*Ipomea* spp.), moonflower (*Ipomoea* spp.), jasmine (*Jasminum*), honeysuckle (*Lonicera*), wisteria (*Wisteria*), hardy kiwi (*Actinidia arguta*), cup and saucer vine (*Cobaea scandens*), hyacinth bean (*Dolichos lablab, Lablab purpureus*), and scarlet runner bean (*Phaseolus coccineus*).

CLINGING STEM ROOTS Plants including climbing hydrangea (*Hydrangea petiolaris*), most ivies such as English ivy (*Hedera helix*) and Irish ivy (*Hedera hibernica*), and also some *Euonymus* spp. climb by utilizing groups of short, stout roots that have the ability to cling to nearly any surface they come into contact with. The roots secrete acids which can damage paint and mortar, thus these vines can be quite troublesome for homeowners.

SO-CALLED CLIMBERS Some plants appear to be climbing as if they were vines but have no mechanism to climb on their own. These plants which include *Bougainvillea* and climbing roses (*Rosa* spp.), simply have very long, flexible stems that are easy to attach to objects giving them the appearance of climbing. Sometimes roses are thorny enough that thorns attach adjacent stems to each other giving a further appearance of climbing.

Leaves

Thigmonastic responses

Physiological responses to touch by leaves is called seismonasty. Probably one of the best known responses is that of leaflet folding. Leaflet folding is observed in a number of species which includes oxalis (*Oxalis* spp.), mimosa (*Mimosa* spp.), Venus fly trap (*Dionaea muscipula*) and many legumes.

When the tip of a mimosa leaf (*M. pudica*), also called the sensitive plant, is touched or heated, the leaflets close in a very orderly fashion down the length of the leaf. The response is rapid, all-or-none and is not limited to the touched leaflet (Simons, 1981; Malone, 1994). The folding is mechanically controlled by specialized organs called pulvini at the base of the leaflets. The folding leaf movement is the result in loss of turgor from extensor cells which are on the top side of the leaflet joint and a stretching of flexor cells, which are located opposite the extensors in the pulvinus. These changes in cell turgor, size and shape are transient and reversible (Braam, 2005). But what causes the turgor of pulvini cells to change? In order to understand the mechanism for turgor changes in pulvinus cells, we must understand the electrical properties of plants.

Electrical potentials in plants

Electrical signals offer a particularly effective means of signal propagation. In studying movement in plants, an understanding of the electrochemistry involved is particularly important, since much of the movement in plants is attributed to changes in cell turgor induced by electrical signals (Volkov and Brown, 2004).

Cell plasma membranes allow electrochemical signals to be passed through the plant over both short and long distances. Changes in the ionic balance inside and outside the plasma membrane generate an electrical current which can move through the plant via an excitation wave or action potential, both of which we will discuss shortly. The rate at which these signals propagate depend on factors such as: (i) the intensity of the stimulus; (ii) previous similar stimuli; (iii) plant health; and (iv) temperature.

Action potentials, which have been studied extensively in the algae *Chara* and *Nitella*, are electrical signals caused by the depolarization of cell membranes. The depolarization is a direct consequence of some sort of stimulus such as touching or wounding. Once the depolarization occurs the electrical signal in the form of an action potential propagates the stimulus message at rates from 0.05 cm/s to 40 m/s. The response to the stimulus can be local or in a distant plant part. Regardless of the distance from the stimulus, the response is nearly always the activation of an enzymatic system, often membrane-bound, that regulates some aspect of plant growth and development.

Plant action potentials have many of the properties of action potentials associated with animals. This includes: (i) the all-or-nothing response; (ii) some minimum threshold for response; and (iii) a refractory period.

There must be a viable system for long-distance transport of electrical signals in plants. A good candidate for such a pathway is the phloem sieve-tube/companion cell array which consists of a continuous line of connected plasma membranes. It may be the 'wire' in a plant responsible for electrical signaling over long distances.

The major chemicals actively involved in the electric system in a plant are relatively few: potassium, calcium, sodium, hydrogen and chloride ions. In general, as the action potential propagates there are large calcium influxes and concomitant potassium and chloride effluxes from cells. These influxes and effluxes are regulated by membrane-bound enzymes which regulate ion flow in and out of the cell through the membrane.

Mimosa leaflet folding

When a mimosa leaflet is open, potassium ions inside extensor cells of the pulvini maintain a turgor to keep the leaflets open. When a leaflet is touched, there is a rapid movement of potassium ions out of the cell with a concomitant efflux of water (Simons, 1981; Fromm and Eschrich, 1988). The water lost may be up to 25% of the initial cell

volume and it can occur within a second (Fleurat-Lessard *et al*., 1997). This stimulation of the extensor cell of a pulvinus causes a transient breakdown in the electrical potential due of the membrane and generates an action potential. The action potential of one cell may stimulate neighboring cells if it is high enough to pass a threshold level (thus the all-or-none response), and the signal is passed down the leaf from cell to cell via plasmodesmata, probably through the phloem, at the rate of about 2cm/s. As the signal moves down the leaf, leaflets fold in a regular and orderly fashion. The membrane potentials eventually return to their original state and potassium re-enters the cells. The cells rehydrate, become turgid, and the leaflet opens. While the membranes are returning to their resting potential, they cannot be stimulated to respond again. This is the refractory period. Once they are back to their resting potential and cells are rehydrated and turgid, leaflets may once again be stimulated to close (Braam, 2005).

The actin network may be involved in perception of the initial signal of touching or heating since chemical inhibitors of actin can block leaflet folding (Fleurat-Lessard *et al*., 1993).

In addition to the short-distance signal transport down a leaf, there may also be long-distance transmission of the signal if the initial stimulus is strong enough. Even though some of the long-distance signal transmission may be electrical, there must be another mechanism since the signal can pass through dead tissue and electricity does not pass through dead tissue very easily. The signal may be changes in hydraulic pressure in the xylem which can be transmitted through dead tissue (Braam, 2005). The long-distance messenger may also be chemical (Schildknecht and Meier-Augenstein, 1990; Ueda *et al*., 2001).

Regulation of stomata movements

Stomata are part of a plant's epidermal system which regulate gas and vapor exchange between the plant and the atmosphere. They are mostly found on leaves, though there can be stomata on green stems, petioles, sepals, petals, and fruit. The discussion of stomata is often limited to their function in leaves, since in other tissues, their regulatory role in growth and development is often limited. There are usually more stomata on the upper side compared with the lower side of dicotyledonous leaves, while monocots usually have the same number of stomata on their upper and lower surfaces. In leaves of floating plants, stomata may be limited to the upper surface.

The functional units of stomata consist of specialized cells called guard cells which are loaded with chloroplasts, and subsidiary cells which lack chloroplasts. When guard cells are turgid, stomata are open, when they are flaccid, stomata are closed. This opening is called the stoma, even though the term is also used to refer to the stomatal complex as a whole. It is the guard cell shape and their cell wall anatomy which provide their shape-changing function.

The cell walls of guard cells are unevenly thickened. The wall away from the pore which forms when turgid (the dorsal wall) is thinner than the wall bordering the pore (the ventral wall). In addition, the cellulose micrifibrils are arranged radially around the cell. The guard cells are attached to each other at each end. Increased turgor in the guard cell causes the dorsal wall to bulge away from the pore and the ventral wall to become concave. The entire cell appears to bow away from the aperture causing the pore between the two guard cells to enlarge. When turgor is lost, just the opposite occurs.

When environmental conditions are favorable for stomatal opening (high light levels (especially blue light), adequate soil moisture) protons (H^+) are pumped out of the guard cells creating an increasingly negative electrical charge inside the guard cell. When the electrical potential reaches a critical level, enzyme channels open allowing potassium ions to flow into the guard cell. To keep the negatively charged guard cell interior, negative ions (either chloride ions or malate ions) enter the cell accompanying the potassium. This increase in potassium and its attendant negative ion decreases the osmotic potential of the guard cell causing water from surrounding cells (subsidiary and epidermal cells) to enter the guard cell, greatly increasing the guard cell turgor. Because the cellulose microfibrils are radially oriented, which prevents the cell from expanding radially, the guard cell elongates. Since the guard cells are attached at both ends and the dorsal and ventral walls are unevenly thickened, the elongating cells bow outwards creating the stomatal pore.

When light is removed from the equation, the proton pumps shut down and potassium and chloride or malate ions leave the cell. The osmotic potential increases, thus water leaves the cell, it becomes flaccid, and the stomatal pore disappears.

During drought or a water deficit, stress occurs, and abscisic acid (ABA) is produced. The concentration of free Ca^{2+} in the cytosol increases due to influx from outside the cell and the release of Ca^{2+} from internal cellular stores such as the endoplasmic reticulum and vacuole. The increase in free calcium concentration causes the chloride ions and inorganic ions to leave the guard cells and stops any further uptake of potassium. In time potassium begins to leave the cell. This increases the osmotic potential causing water to leave the cell. The cells become flaccid and the stomatal pore closes.

The fact that light stimulates the opening of stomata makes sense since light stimulates photosynthesis, and in order for photosynthesis to proceed, carbon dioxide needs to enter the leaf. It also makes sense that carbon dioxide concentration can also regulate stomatal opening and closing. This mechanism is especially important in species where crassulacean acid metabolism occurs (CAM plants) since their stomata are open at night and close during the day.

Stomata open in response to low sub-stomatal cavity carbon dioxide concentrations and close when sub-stomatal levels of carbon dioxide are high. In C3 or C4 plants ('regular' photosynthesis), sub-stomatal carbon dioxide levels increase in the dark since light is not available for driving the photosynthetic machinery. In CAM plants, sub-stomatal carbon dioxide levels remain low at night since carbon dioxide is being fixed to malate.

Low carbon dioxide levels in guard cells (resulting from low sub-stomatal cavity concentrations) stimulates potassium uptake which reduces the osmotic potential of the guard cell. This causes an uptake of water by the guard cells, they become turgid, and the stomatal aperture opens.

Thigmo responses in carnivorous plants

VENUS FLYTRAP (*D. MUSCIPULA*) The Venus fly trap is probably the most widely recognized carnivorous plant. Specialized bi-lobed leaves with three small trigger hairs on the inside surface of each lobe wait for stimulation by an unsuspecting insect (Fig. 4.5). Upon several stimulations of one hair or concurrent stimulation of two or more hairs at a time, the lobes quickly close, entrapping the victim. Hair stimulation induces an electrical signal which causes uneven enlargement of lobe cells resulting in closure within a second (Curtis, 1834; Burdon-Sanderson, 1873; Jacobs, 1954; Jacobson, 1965; Simons, 1981; Fagerberg and Allain, 1991). Attempts to escape further agitate the lobes causing tighter closing and the release of acids and digestive enzymes which results in the victim's demise. This activity supplies the plant with nitrogen in an otherwise nitrogen-poor environment in which they grow.

Fig. 4.5. Venus fly trap (*Dionaea muscipula*) with specialized bi-lobed leaves with three small trigger hairs on the inside surface of each lobe waiting for stimulation by an unsuspecting insect. (Photo by Noah Elhardt, licensed for use under the Creative Commons Attribution-Share Alike 2.5 Generic license.)

SUNDEW (*DROSERA* SPP.) There are many different species within the genus *Drosera* that are called sundew plants. All sundews are characterized by having tentacle-covered leaves which exude sticky mucilage. Insects landing on the leaves become entrapped in the gluey mucilage. Their movement to free themselves induces a slow inward curling of neighboring tentacles further entrapping the prey. Tentacle movement is both thigmonastic and thigmotropic (Lloyd, 1942). The curling inwards and towards the insect seems to be at least partially regulated by auxin levels and tissue sensitivity to auxin, since exogenously applied auxin can trigger the curling response, while auxin blockers can inhibit it (Bopp and Weber, 1981). The sensitivity

of the tentacles is incredible in that they can detect prey weighing less than 1 μg, yet do not respond to the force imparted by raindrops falling on them (Darwin, 1880, 1893).

BLADDERWORT (*UTRICULARIA*) The bladderwort is a rootless plant that grows in aquatic and moist terrestrial environments. Attached to its fine leaves are numerous bladders approximately 3 mm in length held up by thin filaments. Each bladder is a hollow sac, two-cells thick with a valve which remains closed until stimulated by an unsuspecting insect. When the trap is closed, the bladder is under significant negative hydrostatic pressure. Upon stimulation of sensory hairs on or near the valve, the valve opens, sucking in water and the prey that triggered the opening. The prey is then digested within the bladder (Lloyd, 1942). In order to catch more prey, the bladder has to reset itself. This is accomplished by active transport of chloride ions along with accompanying sodium ions into the wall of the bladder. An osmotic gradient is established thereby causing water to leave the lumen of the bladder and enter the bladder wall. Hydrostatic pressure builds up within the wall space, expelling the solution into the outside environment. A bladder can be reset in about 30 min.

General growth responses – thigmomorphogenesis

Gradual changes in growth of plants due to stimuli such as touch or wind is known as thigmomorphogenesis (Jaffe, 1973). These growth changes occur slowly over time and are often not noticed. Shoot thigmomorphogeneis is often characterized by decreased elongation growth with a concommitent increase in radial expansion (Biddington, 1986). Other growth responses include: (i) time of flowering; (ii) dormancy induction and release; (iii) senescence; and (iv) stress resistance (Biddington, 1986).

The signal for these growth changes probably involves the plant hormones as well as intracellular calcium levels (Braam, 2005). Intracellular calcium levels have long been suggested as secondary messengers for many of the stimuli affecting plant growth. Rapid increases in intracellular calcium occur in response to touch or wind stimuli (Knight *et al*., 1991). Reactive oxygen species (ROS) are also thought to play a role in morphogenic signaling (Mori and Schroeder, 2004) and probably interact with calcium in morphogenic regulation (Mori and Schroeder, 2004).

Ethylene has long been implicated in thigmomorphogenic responses. Exogenous application of ethylene can induce thigmomorphogenic growth responses (Goeschl *et al*., 1966; Brown and Leopold, 1972; Jaffe and Biro, 1979; Erner and Jaffe, 1983; de Jaegher *et al*., 1987; Telewski, 1995) and ethylene production occurs after mechanical stimulation (Goeschl *et al*., 1966; Biro and Jaffe, 1984; Takahashi and Jaffe, 1984). However, mutant and inhibitor studies suggest that ethylene is not the primary signaling molecule (Boyer *et al*., 1983; Biro and Jaffe, 1984; Biddington, 1986; Boyer *et al*., 1986; Johnson *et al*., 1998).

A group of genes called touch inducible genes (TCH genes) were discovered in *Arabidopsis* (Braam and Davis, 1990). Since this initial discovery, many more examples of genes induced by touch have been revealed (Ling *et al*., 1991; Perera and Zielinski, 1992; Gawienowski *et al*., 1993; Botella and Arteca, 1994; Botella *et al*., 1996; Mizoguchi *et al*., 1996; Oh *et al*., 1996; Royo *et al*., 1996; Shirsat *et al*., 1996; Eldick *et al*., 1997; Mauch *et al*., 1997; Gilmour *et al*., 1998; Arteca and Arteca, 1999; Gadea *et al*., 1999; Hirsinger *et al*., 1999; Tatsuki and Mori, 1999; Müssig *et al*., 2000; Oufattole *et al*., 2000; Lee *et al*., 2005). Genes that are down-regulated by touch are also known (Lee *et al*., 2005). Calcium binding proteins and wall modifying enzymes are among the major products of touch up-regulated genes. In addition, genes implicated in disease defense responses are also up-regulated by touch.

5 Physiology of Growth in Specific Organs: Flowers, Fruit, and Seeds

In this chapter we will investigate the physiology of some specific growth characteristics of flowers, fruit, and seeds. As in the previous chapter, we will examine some of the mechanisms different plant organs have for growing and prospering as stationary organisms in a dynamic environment.

Flowers

Flowers are extremely important to life on earth. Much of our food is derived from flowers, whether from flowers in their earliest stages of development (such as artichokes, *Cynara cardunculus*), fruit (traditional fruits or vegetables that are botanically fruits), or the final product of their growth, seeds. In addition, flowers provide immeasurable aesthetic value making our lives richer and more colorful. From the bud to the seed, flower growth and development is a carefully orchestrated physiological phenomenon, never ceasing to amaze even the oldest veteran of horticulture.

Plant age and the flowering response – juvenility

All species have a genetically programmed minimal stage of development they must reach before they begin the flowering process. This minimal stage varies considerably among species. We can, however, make some general observations that are applicable to most plants. When a plant is capable of flowering given the appropriate environmental conditions, we say that the plant is 'competent' or 'ripe to flower'. We often measure the 'ripeness to flower' in time or plant stage, with plant stage being more accurate. The usual measure of a plant's stage of development is the number of nodes or leaves it has produced. This measure of competency to flower removes any variability caused by different growth rates due to different growing conditions which exist when measuring competency with time.

When considering perennial plants, we often refer to the age a plant must be before it will flower. Some perennial species will flower in their first year of growth (some conifers) while others require from several years to many decades of vegetative growth before they will flower (Lawson and Poethig, 1995).

Biennial plants require two growing seasons to flower. The first growing season is for vegetative growth, the second is for flowering. The two growing seasons are usually separated by a season of endo-dormancy which must be removed by exposure to cold temperatures (0–5°C). In many biennials, flower bud induction and initiation occur the first season while differentiation and development occur during the dormant and second growing season. In other biennials, all stages of flowering occur in the second growing season, but will only take place after exposure to the previously mentioned cold temperatures.

Most annuals reach a ripeness to flower after a predetermined number of nodes (or leaves) have been produced. Once the adult stage has been reached, the plant will produce a genetically programmed number of flowers. Some species produce a certain number of flowers then die, while others continue to flower until adverse environmental conditions prevent flowering. These differences are important in determining the potential yield of different cultivars, whether for cut flowers, fruit, or seed. With most crops there is a fine balance between vegetative growth and the crop load it can support. Cultivars which produce fewer flowers per plant often produce larger fruit due to lack of competition for photosynthates. In species which tend to produce copious numbers of flowers per plant, flowers or young fruit are often removed from the plant to encourage the production of larger fruit.

Besides the inability to flower, juvenile plants may differ from their adult counterparts in morphology and ease of vegetative propagation (Martin-Trillo and Martinez-Zapater, 2002).

Juvenile plants are often more easily vegetatively propagated than adult plants as juvenile stems often form adventitious roots more readily than adult stems. Adult leaves of English ivy (*Hedera helix*) are shaped differently from their juvenile counterparts and juvenile green bean (*Phaseolus vulgaris*) plants have simple leaves while the adults have compound leaves.

The flowering process

Many plant scientists tend to refer to flowering as a general process. The process as a whole is probably one of the most studied physiological processes in horticulture. It is extremely complex, intricately controlled by genetics and environment. Light and temperature are the two most important environmental factors controlling flowering.

As a process, flowering can be broken down into four major steps: (i) induction; (ii) initiation; (iii) differentiation; and (iv) development. Each step has its own set of responses to light and temperature (Fig. 5.1) (Durner and Poling, 1987). Many scientists fail to distinguish the stages when discussing flowering, which has led to much confusion in the scientific literature. When viewed as a four-step process, flowering is not as complicated as it first seems, even though its control is still quite complex.

Induction

Induction is that process which normally occurs in the leaf as a plant perceives the signal to switch from a vegetative state to a flowering state. This change from vegetative to flowering ultimately occurs at the meristem, but the signal (most often light or temperature) is detected by the leaves. In some plants, including strawberry (*Fragaria* spp.) floral induction can be macroscopically measured by monitoring leaf production (Durner and Poling, 1985). Induction is often monitored to determine effects of different variables on the flowering process.

The number of leaves a plant produces over time is monitored and an average leaf production rate is estimated. Leaf production is fairly constant until there is a sudden marked temporary increase in the rate of leaf production which coincides with the inductive trigger. By being able to determine when induction occurs, we are able to study the factors that influence it more easily.

Once the signal to switch from vegetative to floral growth has been detected, the signal must be transferred to the site of flower production, a meristem. The signal is transferred via the flowering hormone, florigen. More often than not, it is the apical meristem which changes from vegetative to floral, however, axillary meristems may also switch. Once the signal is received by the meristem, the next stage of flowering, initiation, begins.

Initiation

Floral initiation is associated with the observable morphological changes which occur at a meristem as the meristem transitions from leaf to floral production (Durner and Poling, 1985). Actually the change is merely a change in the direction of development as flowers are simply modified leaves. Initiation can be detected by observing changes in morphology of the meristem at the microscopic level. In general, a vegetative meristem is rather pointed and narrow. Once the transition has occurred, the meristem becomes rather flat and broad. The difference is rather easy to observe in most species. Once initiation has been detected at the meristem level, the next stage, differentiation, begins.

Differentiation

There is a fine line between initiation and differentiation. Differentiation is the process in which the various flower parts are formed after initiation, but before macroscopic production has commenced (Durner and Poling, 1985). Differentiation can be monitored via dissection of plant apices under a dissecting scope at magnification from ×10 to ×20. In many biennials and perennials, differentiation takes place over an extended period. In annuals it occurs quickly.

Specific genes have been identified which control: (i) what flower parts are produced; (ii) how many are produced within each flower; and (iii) where they are located (Smyth, 2001; Buzgo *et al*., 2004; Rijpkemaa *et al*., 2010). These genes are probably regulated by environmental cues.

Development

Development refers to the macroscopic production of flowers that are visible without magnification (Durner and Poling, 1985). It is monitored by visual counts of numbers of flowers per plant, inflorescences, etc. Monitoring development closely

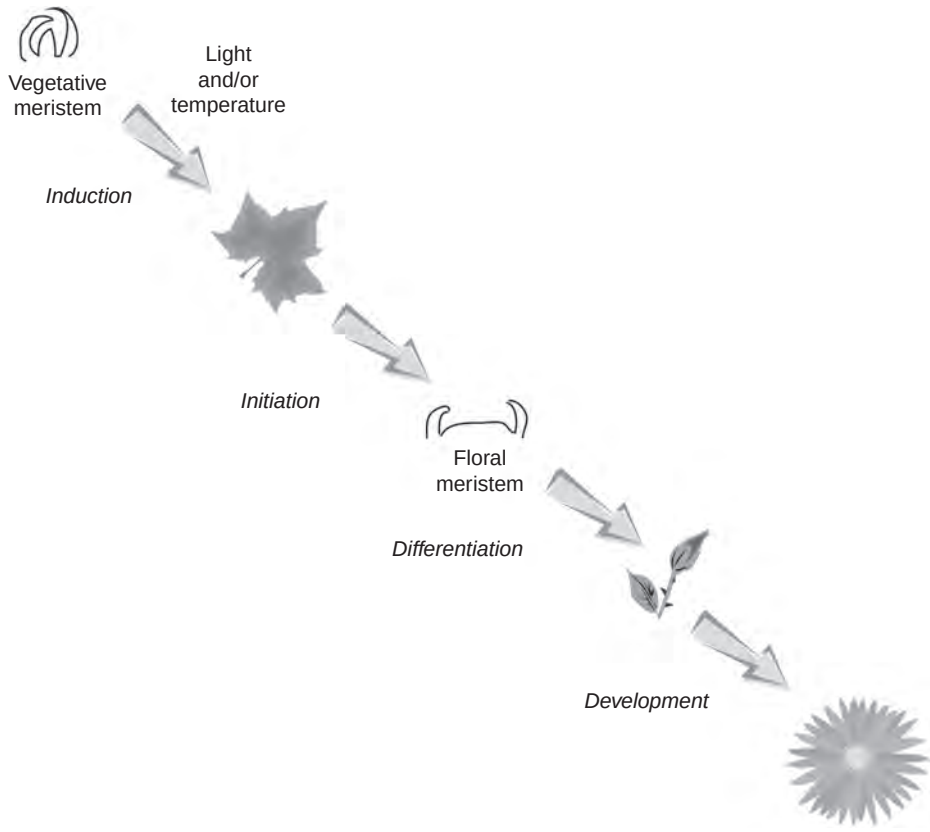

Fig. 5.1. The four stages of flowering. (Leaf, arrow, bud, and flower symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science, ian.umces.edu/symbols/.)

allows careful study of the factors that influence the fruiting potential of many plants. Note the term 'fruiting potential' is just that, potential. Many other steps are involved in the production of fruits and seeds after the production of flowers.

Reproductive mechanisms

Much of our horticultural production relies on adequate sexual reproduction in plants. It is therefore important to understand the underlying basic biology of sexual reproduction as well as the physiology behind the time of bloom. The reference for the technical details that follow is Evert and Eichorn (2006).

The alternation of generations

The life cycle of flowering plants consists of two separate generations, sporophyte and gametophyte, which alternate back and forth as plants sexually reproduce (Fig. 5.2). The generation most people recognize is the sporophyte generation, the more highly visible of the two. The sporophyte generation is, as the name suggests, the generation that produces spores. The gametophyte generation is the generation that produces the gametes involved in sexual reproduction, the egg and sperm cells. The gametophyte generation is small and actually takes place in flowers of a sporophyte plant. We'll look at each generation in detail.

SPOROPHYTES The sporophyte generation is what we see when we see a 'normal' plant. It is diploid, meaning it has two sets of chromosomes. The smallest sporophyte plant is the single-celled diploid zygote that results from the fertilization of an egg with one of the sperm cells from a pollen grain. Its cells undergo mitosis resulting in growth.

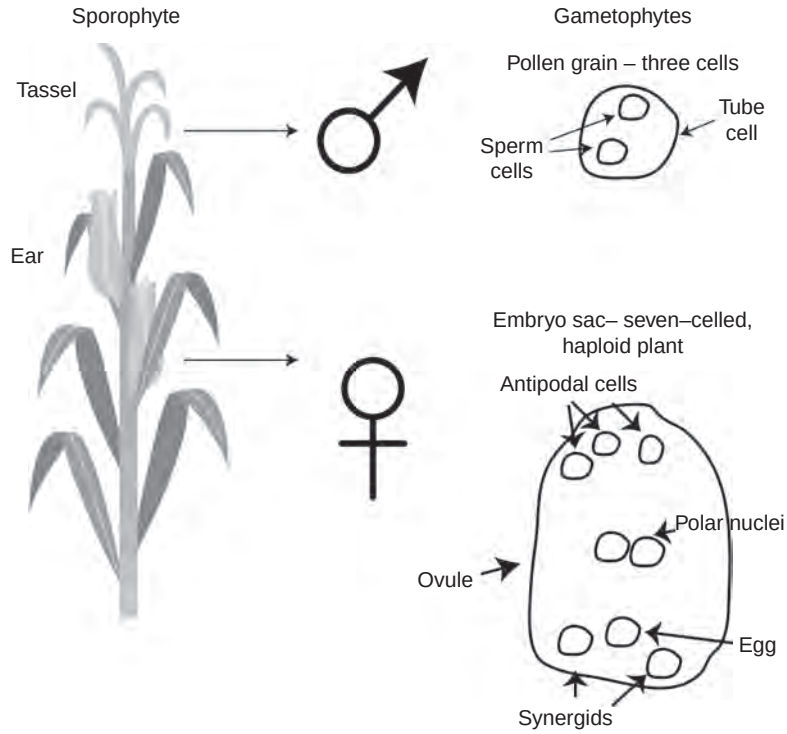

Fig. 5.2. The alternation of generations. (Corn and gender symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science, ian.umces.edu/symbols/.)

Ultimately this diploid sporophyte, which over time has acquired the multi-cellular appearance of a 'normal plant', will undergo meiosis in certain tissues (the anthers and ovules of flowers) to produce two different types of gametophyte plants, the male and female gametophytes.

GAMETOPHYTES Gametophytes are multi-cellular haploid organisms (plants) residing in the flowers of sporophyte plants. The male gametophyte is called the pollen grain and is derived from a microspore mother cell which is found in the anther of a flower. There are many microspore mother cells in any given anther. Each of these cells divides to form four haploid microspores. Each microspore develops into a pollen grain which consists of a large vegetative cell, called the tube cell, inside of which lives a smaller generative (germ) cell. The haploid germ cell divides by mitosis to produce two sperm cells. Thus the pollen grain is a three-celled haploid male gametophyte plant made up of two sperm cells living inside a tube cell.

The female gametophyte is a seven-celled haploid plant which develops from a megaspore. A diploid megaspore mother cell located in the ovule of a sporophyte plant undergoes meiosis to form four haploid megaspores, three of which disintegrate. The remaining haploid megaspore undergoes mitosis four times to form eight haploid cells. One of these cells is the egg cell, which if successfully fertilized will become part of the new sporophyte. The egg cell is flanked by two cells called synergids. These three cells (the egg plus two synergids) sit at the micropyle end of the ovule (this is the end of the ovule with space for the pollen tube to grow through during pollination). Three of the remaining five haploid cells sit at the antipodal end of the ovule (the end away from the micropyle) and they are called antipodal cells. The remaining two haploid cells fuse into a single cell with two polar nuclei and this cell sits in the middle of the ovule. Thus we've accounted for all eight haploid cells which formed from a single megaspore. They have now developed into a seven-celled haploid female gametophyte plant.

Upon pollination and subsequent fertilization (both will be covered shortly), the new sporophyte generation begins as a single-celled zygote which eventually grows into an embryo. The new sporophyte plant we see develops from this embryo. Pretty amazing, isn't it?

Controlling sexual reproduction

Sexual reproduction in plants is important for genetic diversity. There are a number of mechanisms which have evolved in plants that impart at least some degree of control on plant sexual reproduction. Many of these mechanisms ensure that self-pollination does not occur. Forcing pollination by another individual reduces inbreeding which could lead to homozygosity, which often leads to expression of undesirable traits or the lack of expression of desirable traits. Some mechanisms which encourage heterozygousity include: (i) dioeciousness; and (ii) asynchronous time of bloom on monoecious plants.

Plant types

Although many species (85%) of plants have flowers that are perfect (i.e. include both male and female gametophytes residing in the same flower), there are many species which employ a different approach to sexual reproduction (Yampolsky and Yampolsky, 1922). This mechanism is such that separate male and female flowers exist. If both male and female gametophytes are produced on the same plant but they reside in separate flowers, the species is monoecious. About 7% of plant species are monoecious (Dellaporta and Calderon-Urrea, 1993). Good examples of this are corn (*Zea mays*) (Fig. 5.3) and squash (*Cucurbita maxima*) (Fig. 5.4). When a species' male and female gametophytes reside on separate plants, that species is dioecious. About 6% of plant species are dioecious (Renner and Ricklefs, 1995). A popular example of this type of plant is holly (*Ilex opaca*). In dioecious species, male and female plants must be close enough together such that pollen is easily transferred from the male plant to the female plant if sexual reproduction is to be successful. If fruit are a nuisance, as is often the case in landscaping situations, plants should be screened to include only male plants to ensure that fruit won't be produced. Selecting all-female plants may not be effective, since there may be male plants of the same species on neighboring properties.

Time of bloom

Time of bloom of two different plants within the same species can be synchronous or asynchronous. Some cultivars, especially in fruit crops, are self-incompatible with respect to pollination, thus they

Fig. 5.3. A typical corn (*Zea mays*) plant with separate flowers: (a) male tassel, top; and (b) female ear, on stalk.

Fig. 5.4. The female flower of a typical winter squash (*Cucurbita* spp.) plant. Female flowers are easily identified due to the often prominent ovary subtending the corolla. Male flowers conspicuously lack the ovary.

require pollen from a different cultivar of the same species. Synchronous bloom among cultivars is important for effective pollination in these crops. Asynchronous time of bloom is an effective mechanism for ensuring that cross-pollination occurs to encourage heterozygousity.

This last macroscopic step in the flowering process is under both genetic and environmental control. Once flowering occurs, two more steps, often microscopic, occur: (i) pollination; and (ii) fertilization. We will first explore the control of bloom time then look at pollination and fertilization.

GENETIC CONTROL OF BLOOM – LOW-CHILL CUTIVARS Every species has an internally programmed time to bloom, thus the ultimate control is genetic. The appropriate environmental conditions must exist for the genetics to be expressed, thus environment is secondary, yet equally important. Many deciduous species require exposure to a period of relatively cold temperatures (0–5°C) between growing seasons before they will resume growth. This is called the chilling requirement. Once the chilling requirement has been fulfilled, plants begin growth when exposed to warm temperatures as measured via heat units. The physiology of chilling and heat units will be covered extensively in Chapter 9, this volume.

Some cultivars of peach (*Prunus persica*), apple (*Malus domestica*), and blueberries (*Vaccinium* spp.) have a greatly reduced chilling requirement when compared with other cultivars within their respective species. They are called 'low-chill' cultivars. These cultivars are often used for commercial production in regions which normally do not receive enough cold weather to fulfill the 'normal' chilling requirement. They should not be used in areas where significant chilling accumulation occurs, since their chilling requirement would be fulfilled early in the dormant season. Any exposure to warmer weather would then lead to premature heat-unit accumulation and spring-like growth which would probably be injured or killed with the return of harsh temperatures. The low-chill character is genetically controlled and has been quantified for many commercial cultivars.

GENETIC CONTROL OF BLOOM – LATE-BLOOMING CULTIVARS While the low-chill cultivars have bloom times regulated primarily by their exposure to cold temperatures, there is a group of apple cultivars which tend to bloom much later than the average cultivar due to a greater heat-unit requirement. The greater heat-unit requirement may be due to an absolute increase in the amount of heat needed for development or it could be a result of decreased sensitivity to lower temperatures. In the latter case, fewer heat units would accumulate at lower temperatures while in the former, more heat units must accumulate for bloom to occur.

ENVIRONMENTAL CONTROL OF BLOOM All species have an inherent genetic control of the time of bloom which may be modified by the environment. In general, species have a genetically controlled chilling and heat-unit requirement which is constant and quantifiable. Time of bloom can be modified by altering the chilling/heat required for bloom or by altering the chilling/heat accumulated. Accelerated bloom is usually not a desirable trait for most field-grown horticultural crops. Accelerated bloom may be desirable for out-of-season production in greenhouses or high tunnels. Most often our attempts to modify the time of bloom are attempts at delaying bloom to avoid frost or freezing injury to fruit blossoms. In certain regions of the world, frost or freezing injury may result in 100% crop loss. Methods that have been studied for delaying bloom and the physiology behind the plant response will be discussed in Chapter 9, this volume.

Pollination

While the general mechanisms for regulating sexual reproduction have been discussed, specific control

of the process lies in pollination and fertilization. Both processes are highly regulated at the physiological level.

MECHANISMS OF POLLINATION Sexual reproduction is a highly specialized process closely regulated anatomically and physiologically. Only similar genomes can cooperate to form a new individual via sexual reproduction. Various 'security measures' have evolved in plants to ensure only compatible gametes unite.

Successful pollination requires several steps. Initially, pollen must land on the stigma of a pistil. The transfer of pollen from the anther to the stigma is accomplished either via wind or insects. Once the pollen grain lands on the stigma, it must germinate, grow into the stigma then down through the style into the locule of the ovary towards the awaiting egg. Pollen tube growth through the style is accomplished with the weakening of stylar tissue by enzymes produced by both the pollen tube and the style. These enzymes are only produced when compatible pollen attempts to grow through the style. Once the pollen tube reaches the egg, the egg and one of the sperm cells from the pollen grain unite to form the zygote. The zygote eventually develops into the embryo.

Pollination is accomplished by either biotic or abiotic means. Approximately 10% of flowering plants are pollinated abiotically by either wind (anemophily) or water (hydrophily), with wind pollination accounting for 98% of abiotic pollination. Abiotically pollinated flowers are often inconspicuous and non-showy.

Nearly 90% of all flowering plants require a pollinator, that is, an organism that can move pollen from the anther to the stigma. The plant from which the pollinator takes the pollen is called the pollinizer. Most of the 200,000 or so pollinators are insects. Pollination by insects is called entomophily and is accomplished by organisms such as: (i) bees, wasps, and ants (*Hymenoptera*); (ii) beetles (*Coleoptera*); (iii) moths and butterflies (*Lepidoptera*); and (iv) flies (*Diptera*). Most plants pollinated by insects have colorful, strongly scented flowers.

Pollination by vertebrates is called zoophily, and is accomplished by birds and bats. Plants that are pollinated by bats or moths tend to have white petals with strongly scented flowers. Plants pollinated by birds usually have red petals and not much of a scent.

INTERACTIONS OF POLLEN AND STIGMA/INCOMPATIBILITY A pollen grain is a three-celled haploid male gametophyte plant consisting of two sperm cells living inside a tube cell. Pollination occurs when the haploid pollen grain lands on the diploid stigma surface. If the stigma is covered in mucus, it is called a wet stigma. If it is covered in cutin, it is called a dry stigma. The pollen grain can be compatible or incompatible with the stigmatic surface. Compatibility will result in pollen tube growth and potential fertilization of the egg cell. Pollen tube growth and subsequent fertilization will not occur if there is incompatibility.

Pollination and subsequent fertilization between genera is relatively rare. In general, pollen from different genera is not compatible primarily because of differences in chromosome number. However, there are some notable exceptions to this general rule. The showy ornamental plant × *Heucherella tiarelloides*, is an intergeneric hybrid of *Heuchera sanguinea* × *Tiarella cordifolia*. Many common orchids are intergeneric hybrids, as is the leyland cypress, × *Cupressocyparis leylandii*.

Within a genus, interspecific pollination and fertilization can occur resulting in interspecific hybrids. French–American hybrid grapes (*Vitis*) are well-known examples of this. Other examples include: (i) loganberry (*Rubus* × *loganobaccus*), a hybrid of blackberry (*Rubus ursinus*) × raspberry (*Rubus idaeus*); (ii) peppermint (*Mentha* × *piperita*), a hybrid of spearmint (*Mentha spicata*) × water mint (*Mentha aquatica*); (iii) tangelo (*Citrus* × *tangelo*), a hybrid of mandarin orange (*Citrus reticulata*) × pomelo (*Citrus maxima*) or grapefruit (*Citrus* × *paradisi*); (iv) triticale (× *Triticosecale*), a hybrid of wheat (*Triticum* spp.) × rye (*Secale cereale*); and (v) grapefruit (*Citrus* × *paradisi*), a hybrid of pomelo (*C. maxima*) × sweet orange (*Citrus sinensis*).

Within a species, crosses between cultivars may or may not occur readily due to one of several types of incompatibility. One type of incompatibility is called self-incompatibility. Nearly 60% of angiosperm species have some form of self-incompatibility (Hiscock and Tabah, 2003). In self-incompatible species, the pollen of a plant will not develop a pollen tube on the stigma of the same plant. Pollen from another plant of the same species will develop a pollen tube, as long as it does not carry the same allele for incompatibility as the stigma on which it lands. The self-incompatibility ensures cross-fertilization and is a barrier to inbreeding and the homozygosis it causes.

Gametophytic self-incompatibility is regulated by a single gene (SI) with multiple alleles (SI1, SI2, SI3, SI4 . . . S*i*n) and is the most common (Hiscock

and Tabah, 2003). The incompatibility reaction occurs whenever the pollen and the stigma have the same alleles. Remember that the pollen is haploid with one allele while the stigma is diploid with two alleles. Effective pollination and fertilization may only occur if the alleles of the pollen and stigma are different.

Sporophytic self-incompatibility occurs when some molecular component in the pollen is repulsed by the stigma which prevents effective germination of the pollen tube.

TYPES OF POLLINATION Plant systems include several different types of pollination. Self-pollination occurs when the pollen of a plant lands on its own stigma and germinates into a pollen tube. Cross-pollination occurs when pollen from one plant lands on the stigma of another (of the same species, of course). In addition to self- and cross-pollination, there are the controlled crosses of hybrid development. This type of pollination occurs when the pollen of a specific cultivar is used to pollinate the stigma of a second specific cultivar to produce seed of a hybrid cultivar. The process is usually labor intensive and also is the culmination of many years of work by one or more plant breeders. That is why hybrid seed is more expensive than open-pollinated cultivars. Successful pollination does not ensure fertilization with the seed and fruit development that normally follows. There are a number of other fates that may await the pollen tube.

STERILITY There is a difference between sterility and incompatibility. Sterile plants fail to produce spores or gametes or those that are produced are abnormal. In systems of incompatibility, the male and female gametes are normal and functional but will only produce a zygote with a compatible mate.

Sterility is a reproductive system in plants where plants fail to produce functional gametes. Male sterility is much more prevalent or at least more widely known than female sterility. Perhaps it is more prevalent due to the fact that the male sporophyte and gametophyte are less protected from the environment compared with their female counterparts and exposure to the elements may induce greater chances of sterility. It could also be that the condition is simply more widely recognized since it is easier to detect male sterility because of the much greater number of male gametophytes produced when compared with the number of female gametophytes produced. In addition, it is easy to assay male sterility with staining techniques. Detection of female sterility requires crossing.

MALE STERILITY Male sterility arises from spontaneous mutations in nuclear or cytoplasmic genes and appears in a number of different ways (Schnable and Wise, 1998; Budar and Pelletier, 2001). It can appear as: (i) an absence or malformation of the stamen; (ii) lack of male flowers; or (iii) a failure of flowers to produce anthers. If anthers are produced, microsporogenesis may be abnormal resulting in deformed or non-viable pollen. Pollen may form but fail to mature normally, thus preventing germination upon pollination. Finally, totally viable pollen may be produced but is not released due to a failure of anther dehiscence.

We will focus our discussion on male sterility since it is more prevalent than female sterility. Male sterility is classified as phenotypic or genotypic (Fig. 5.5).

PHENOTYPIC MALE STERILITY Phenotypic male sterility occurs as: (i) structural male sterility; (ii) sporogenous male sterility; or (iii) functional male sterility.

Structural male sterility occurs when there is some structural malfunction of the male sex organs. Sporogenous male sterility occurs with a malfunction of the anthers during pollen development such that very little pollen is formed or the pollen that is formed is defective. Functional male sterility occurs when functional pollen is produced by the anthers but some barrier to fertilization occurs. These barriers include: (i) lack of anther dehiscence and subsequent lack of pollination; (ii) pollen clumping such that it isn't released; and (iii) excessive pollen tube growth.

GENOTYPIC MALE STERILITY There are three types of genotypic male sterility: (i) genic; (ii) cytoplasmic; and (iii) genic-cytoplasmic (Budar and Pelletier, 2001; Fig. 5.5).

Genic male sterility involves nuclear genes and follows laws of Mendelian inheritance (Kaul, 1988; Chaudhury, 1993). In most cases it arises as a spontaneous mutation. This type of male sterility has been identified in about 175 species and is controlled by a single recessive gene in most of the species in which it occurs. Multi-gene control also occurs in some species.

Cytoplasmic male sterility is controlled by cytoplasmic rather than nuclear genes and follows non-Mendelian inheritance. This form of male sterility

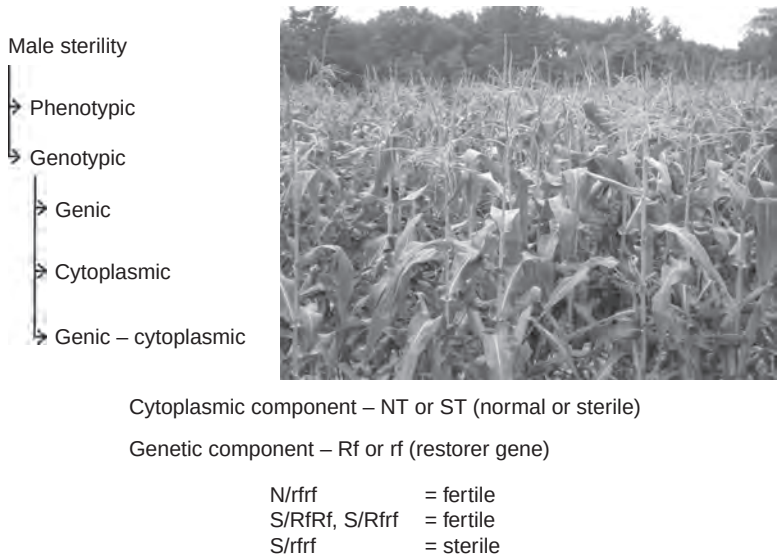

Fig. 5.5. Cytoplasmic and genetic control of male sterility.

is not very common, it is inherited maternally and there are normal (N) and sterile (S) cytoplasms (Kaul, 1988).

In cases of genic-cytoplasmic male sterility, both nuclear and cytoplasmic genes are involved (Schnable and Wise, 1998; Skibbe and Schnable, 2005). Normal and sterile cytoplasms combine with genes which restore fertility (Rf) which are distinct from genic male sterility genes. The Rf gene is required to restore fertility with sterile cytoplasms. The following combinations of cytoplasm and nuclear genes can occur:

- N/rfrf which is fertile.
- S/RfRf or S/Rfrf which are fertile.
- S/rfrf which is sterile.

Mutations of the restorer gene are frequent, thus the combination of normal cytoplasm with RfRf genes is best to maintain stable fertility. The best known system for this kind of male sterility is in corn (*Zea mays*) (Skibbe and Schnable, 2005). Several sterile cytoplasms occur in corn, among which T, S and C are the most well known. Since T cytoplasm was involved in the great corn blight epidemic of the 1970s in the USA, let's take a look at what happened a little more closely.

The T (T is for Texas) cytoplasm is very stable under many environmental conditions (Levings, 1990). It works by preventing anther exertion with pollen abortion. Unfortunately, plants with the T cytoplasm are highly susceptible to race T of southern corn leaf blight (*Cochliobolus heterostrophus* = *Bipolaris maydis*). This race of southern corn leaf blight is incredibly vigorous and virulent allowing it to spread rapidly through any population with the T cytoplasm.

But why would anyone use male sterility in crop production in the first place? Male sterility makes the production of hybrids very efficient since emasculation of the female parent is not needed. If the female parent has cytoplasmic male sterility, no viable pollen will be produced, thus there is no need to remove anthers to get a hybrid. Nearly all commercially produced corn cultivars are hybrids, thus male sterility is widely used in hybrid corn seed production.

In the 1960s there was widespread use of the T-cytopolasmic male sterility system in hybrid corn seed production, which meant that many of the hybrids used for field production had the T cytoplasm and were therefore extremely susceptible to race T of *C. heterostrophus*. Mitochondria in T cytoplasm are extremely sensitive to a toxin (β-polyketol also called T-toxin) produced by the fungus.

A gene in T cytoplasm (T-urf13) confers both male sterility and mitochondrial sensitivity to fungal toxins. This gene is only present in T cytoplasm

and encodes a large protein on the inner mitochondrial membrane. The fungal toxin interacts with this protein and causes abnormal plasma membrane permeability (Levings, 1990).

The urf13 gene causes male sterility by causing the tapetum, a layer of nutritive cells in the pollen sac, to degenerate during microsporogenesis. This disrupts pollen development leading to pollen abortion. The urf13 protein is only toxic in anther cells, even though the protein can be found throughout the plant. The protein may cause a minor reduction in plant vigor and yield, but for the most part, has little other effect on the plant.

Basically the gene causes mitochondrial dysfunction in anthers of corn. But why only in the anthers even though it's found all over the plant? Anthers have an exceptionally high energy requirement during pollen development. There are 40 times more mitochondria present in tapetum cells and 20 times more mitochondria in sporogenous cells during pollen development compared with other cell types. Any perturbation in energy production during pollen development could have lethal consequences. Tapetum cells are evidently extremely sensitive to slight changes in mitochondrial activity. When multiplied by 40, this severely impacts pollen production. Other cells are evidently not nearly as sensitive, thus there are no observed deleterious effects of the urf13 protein.

There are two genes, Rf1 and Rf2, involved in restoring fertility to T-cytoplasmically sterile corn plants. Sterility is eliminated in plants with a dominant set of these alleles even though they continue to produce the urf13 protein. Fertility is restored prior to mitosis in the anther, thus this type of fertility restoration is called sporophytic. Even though these genes restore fertility, they do not impart full resistance to corn blight. Only normal cytoplasm confers resistance.

The Rf1 gene is specific for restoring fertility. The Rf2 gene is important in the production of aldehyde dehrogenase, an enzyme needed to detoxify accumulated ethanol and acetaldehyde in cells. The highest levels of Rf2 protein are found in the tapetum. When mitochondrial activity is negatively affected by male sterile cytoplasm, Rf2 might scavenge the toxic ethanol and acetaldehyde produced by alternate energy production pathways in the cells of the tapetum induced by the dysfunctionality of the mitochondria. Thus even though the mitochondrial activity is adversely affected by the male sterile cytoplasm, the Rf2 gene restores pollen fertility by getting rid of toxic ethanol and acetaldehyde. If the Rf2 isn't present, the ethanol and acetaldehyde build to lethal levels in tapetum cells, thus there is no viable pollen.

Other cytoplasmic male sterility systems have been identified in green beans (*P. vulgaris*), sorghum (*Sorghum* spp.), beet (*Beta vulgaris*), carrot (*Daucus carota*), onion (*Allium cepa*), petunia (*Petunia* × *hybrida*), *Brassica napus*, rye (*Secale cereale*), sunflower (*Helianthus annuus*), and wheat (*Triticum* spp.).

DICHOGAMY Dichogamy is the separation in time of maturation of male and female flower parts such that self-pollination is avoided. Protandry occurs when the anthers mature before the pistils. Many species in the family *Compositae* and family *Leguminosae* exhibit protandry with pollen released from anthers before the stigma in the same flower is receptive. Corn (*Zea mays*) is a protandrous, monoecious, diclinous plant. (Diclinous species are those species which have separate male and female flowers on the same plant.) The tassel is the male flower while the ear is the female flower. The silk on an ear of corn are the styles of the pistil. Sweet corn is harvested as soon as the silks begin to whither and turn brown. At that point, the kernels have had just enough time to develop so that they are tender and sweet when harvested.

Protogyny occurs when the stigma becomes receptive before the anthers in the same flower shed their pollen. Many species of the family *Rosaceae* and family *Cruciferae* are protogynous.

Both protandry and protogyny may occur within a flower (intrafloral dichogamy) or among flowers in diclinous species (interfloral dichogamy). Sometimes the maturation of the two sexes are completely separated (complete dichogomy) while in other cases sexual maturity overlaps (incomplete dichogamy). Dichogamy is often considered an evolutionary mechanism for reducing the frequency of self-pollination and inbreeding. If this is the reason (if there is one) for dichogamy to exist, it is a redundant feature in many species, since many species that are dichogamous are also self-incompatible. Besides separation in time, pollen and stigmas can be separated in space. Herkogamy, the spatial separation of pollen and stigma, does not necessarily prevent self-pollination. All monoecious plants exhibit herkogamy.

EFFECTIVE POLLINATION PERIOD Whether or not pollination leads to fertilization also depends

on the effective pollination period. The effective pollination period is the length of time viable pollen has the chance of being deposited on a receptive stigma and germinating. The simplest estimate is length of ovule longevity minus the number of days it takes the pollen tube to grow down the style. Estimates have been made for many crops and varies from less than 24 h to as long as 1 week or more. Estimates of the effective pollination period must take into consideration: (i) stigmatic receptivity; (ii) pollen viability; (iii) pollen tube growth; and (iv) ovule longevity. Dichogamy adds further complexity to the situation, as there may be lags between anthesis, ovule maturation, stigma receptivity, and pollen tube growth.

TEMPERATURE EFFECTS ON POLLINATION Temperature affects pollination by influencing pollen dispersal, pollen growth, and pollinator activity. In general pollen will not germinate at low temperatures (below 4°C) and pollen tube growth is slow below 5°C.

Insect pollinators

Many economically important crops are pollinated by insect vectors (McGregor, 1976). Of all the insects capable of pollination, bees are the most important vectors for horticultural crops. While not strictly a physiological issue, pollination by insects is so crucial to the survival of many species, including our own, a discussion of bee biology and function is justified here.

BEE BIOLOGY AND POLLINATION There are over 19,000 species of bees worldwide! Bees are members of one of seven families in the superfamily *Apoidea*, order *Hymenoptera*, class *Insecta* in the animal phylum *Arthropoda* (Winston, 1987). All bees other than the domesticated honeybee (*Apis mellifera* L.) are considered wild. Though bees are incredibly important for the pollination of many worldwide crops, few species have been utilized for such purposes. Besides honeybees, only five other genera have been domesticated for their assistance in pollinating mankind's crops. These include: (i) leafcutter bees (*Megachile pacifica*); (ii) alkali bees (*Nomia melanderi*); (iii) Osmia bees (*Osmia* spp.); and (iv) bumblebees (*Bombus* spp.) (Mader *et al*., 2010).

Bees feed their young pollen and nectar, thus they forage for food on flowers. While doing so they transfer pollen from anther to stigma and from flower to flower and *voila*, pollination!

Contrary to popular belief, many bees do not sting. In those that do, the sting is accomplished by modified ovipositors in females or exposed genitalia in males. Besides the obvious, you can determine the sex by looking at antennae. Male bees have antennae with 13 segments while female antennae have 12 segments.

Bees are solitary, gregarious or social. Solitary bees are characterized by females who construct one single-celled nest at a time. The female bee deposits an egg then seals the cell, all with no help from any other bee. Gregarious bees are solitary individuals but they tend to nest close to each other. Social bees live together in hives and work cooperatively with each other to maintain their community. The main life activity of bees is foraging for nectar and pollen. Most bees forage for pollen and nectar from as little as a few hundred feet to as much as 25 km from their nest depending on species.

HONEYBEES The bee most common to horticulturists is the European honeybee, *Apis mellifera* L. There are a number of varieties of European honeybees that have been bred for honey production, disease resistance, pollination efficiency, and temperament. Extensive cross-breeding can occur and only queens from commercial breeders should be used when reinvigorating a hive.

Some of the more prevalent varieties or stocks of honeybees include the German, Italian, Carniolan, Caucasian, Buckfast, and the Russian bee.

The German or black bee (*Apis mellifera mellifera* L.) is a hardy, aggressive honeybee which has been all but wiped out in North America by disease.

The Italian bee (*Apis mellifera ligustica*) is favored by beekeepers for their excellent honey production and long brood life. They tend to consume surplus honey if it is not removed from the hive in a timely manner and they also tend to steal honey from neighboring, weaker hives. They are also less defensive and prone to diseases than the German race. The Buckfast bee, which is a descendant of the Italian race, was developed by a monk to thrive in the cold, wet conditions of the British Isles.

The Carniolan bee (*Apis mellifera carnica*), also from middle Europe, is favored for its rapid colony growth early in the spring. They are docile, build very nice wax combs and do not rob other hives of honey. They do have a tendency to swarm.

The Caucasian bee (*Apis mellifera caucasica*), from Eastern Europe, are prized for their long tongues that can reach nectar other honeybees can't. They are docile, but very slow to get started in the spring. They also tend to use excessive propolis (a sticky substance) when building their hives, making the hives hard to manage.

The Russian bee, originated from Russia near the Sea of Japan. It is resistant to the varroa mite which has devastated honeybee hives around the world. They have good, clean hives and are also resistant to the tracheal mite. While most bees have queens only during hive replacement or swarming, Russian bees have queen bee cells present in their hives at all times. Cross-contamination with other strains greatly reduces their resistance to varroa mites.

Some other stocks include: (i) the Minnesota Hygienic stock, known for its hive-cleaning character; (ii) the SMR (Suppression of Mite Reproduction) stock, which is a collection of mite-resistant stocks used in breeding projects; and (iii) the Cordovan stock, a very light yellow Italian bee.

Many commercial hybrid stocks have been generated by bee breeders, including the Midnight, the Starline, the Double Hybrid and the Smart strains. The Midnight strain is a cross of the Caucasian and Carniolan stocks which was developed to decrease the excessive propolis use of the Caucasians and reducing the swarming tendency of the Carniolans while maintaining the docility of both. The Starline was developed for its exceptional honey production. The Double Hybrid is a Midnight and Starline cross. The Smart strains are crosses of previously mentioned stocks with the SMR line.

In order to understand how honeybees do what they do best, make honey and pollinate, you must understand their life cycle and social nature. There are three levels of bees in the honeybee social scene: (i) the queen; (ii) the drones; and (iii) the workers. The queen lays eggs and she regulates whether or not the egg that is laid is fertilized or not. Fertilized eggs become females while non-fertilized eggs become drones. Females are workers unless as larvae they were fed copious amounts of royal jelly by workers, when they would then be virgin queens.

A queen can lay as many as 1000 eggs/day in hexagonal cells of the honeycomb, one egg per cell. Eggs hatch into larvae in 3 days and are fed royal jelly for 2 days. Workers must eat tremendous amounts of pollen and nectar to produce enough royal jelly to feed the developing larvae. On the third day of development, larvae destined to be workers are fed honey, pollen, and water while those destined to be queens are fed royal jelly for their entire larval life. Larvae molt five times to become pupae. During the larval stage, each larva is fed nearly 10,000 times and their weight increases 1500-fold. Development time in the larval stage varies with their adult destiny:

- Queens are larvae for 5.5 days.
- Workers are larvae for 6 days.
- Drones are larvae for 6.5 days.

Once they become pupae, the cells are capped by the workers. The length of pupation varies from 7.5 days for queens, 14.5 days for drones and 12 days for workers. Thus the time from egg laying to emergence as an adult is approximately 15 days for the queen, 24 days for the drones, and 21 days for workers.

An active honeybee colony typically consists of 50,000–60,000 workers, 500–1000 drones and one queen who typically lives for 4 or 5 years. Workers collect pollen and nectar from flowers sometimes from as far as 4.8 km away from the hive. Each worker typically makes ten trips a day. Pollen is fed to larvae or stored in cells of the hive. The nectar is regurgitated along with an enzyme (invertase) into honeycomb cells where it is converted to honey and evaporated. Wax produced as small flakes is chewed and reshaped to form the honeycomb by workers. While the workers are busy constructing the hive and gathering nectar and pollen, the colony continues to grow as the queen continues to lay eggs and workers feed the brood. A worker normally lives for only 1–2 months.

After emerging from their pupal stage, worker bees are cared for by older worker bees for 4 days. After 4 days, adults work around the hive for about 21 days. During that time they may clean the hive, build more honeycombs, feed larvae, process honey or cool the hive by fanning. After about 21 days, workers switch to pollen and nectar gathering, which they do for about 20 days before they leave the hive to die.

Collected pollen is brought back to the hive and fed to larvae or stored for later use. Nectar is regurgitated to younger 'hive workers' where it is processed in their digestive system and regurgitated into storage cells where it ripens for 5 days. After 5 days it is honey and the cell is capped for storage. It takes the nectar from about 5 million flowers to make 1 pint (473 ml) of honey.

If a new queen is needed due to death or expansion of the colony, a special wax cell is built around seven or eight fertilized eggs. The eggs and subsequent larvae are fed royal jelly for their entire larval life which causes them to become fertile females (queens) rather than workers. The first queen to emerge from her pupal case kills all of her sister queens and sometimes her mother as well. Within 2 weeks of her emergence, the new queen will fly off and mate with up to 10 drones. Drones who mate die immediately. The queen will store their sperm in a special organ called a spermatheca. The sperm remains viable for up to 4 years and she will never mate again. When she returns to the hive, she will lay up to 1500 eggs/day. In 2–4 years, she will have used up all the stored sperm and begin laying unfertilized eggs which will become drones. Workers will raise one or two new queens from the last of the fertilized eggs. Commercially, beekeepers often replace the queen each year to maximize hive productivity.

If the hive has become too large, the queen will leave the colony with some worker bees to find a new home in the often maligned process of swarming. Swarming usually occurs in the spring. The initial departure of the queen with about 60% of the workers is called a prime swarm. One to five afterswarms with virgin females may occur. In the process of swarming, colonies reproduce, one original hive becoming two or more new hives.

Commercial beekeepers normally do not want their colonies to swarm. They therefore control swarming by removing brood honeycombs from the hive to make sure the colony doesn't get too big. The removed combs can be sold or used to create new hives.

As the growing season ends and winter approaches in the temperate zone, honeybees begin preparing to overwinter. Drones that did not mate are expelled from the hive and die. The remaining queen and workers do not hibernate, they cluster.

As air temperatures drop below about 14°C, honeybees cling to each other on combs inside the hive forming a cluster inside the hive (Owens, 1967). This cluster keeps the temperature inside the hive warm regardless of the outside temperature. The cluster moves within the hive to reach honey as needed. Some bees die during the cold season. In warmer climates, colonies may not cluster but rather, continue to forage and make honey while the queen lays eggs.

Once the queen resumes egg laying towards the end of the winter, the colony must keep the temperature of the cluster between 34°C and 37°C. If the temperature dips below this range, the brood will die.

COLONY COLLAPSE DISORDER In 2006, an alarming number of honeybee hives in the USA began to die off for no apparent reason. Oddly enough, no dead bees are ever found in or near the hive, and a queen and the brood are still alive. This mysterious problem was given the name 'colony collapse disorder' (vanEngelsdorp *et al*., 2009).

The number of managed honeybee hives in the USA has dropped from 5 million in the 1940s to 2.5 million presently. To gain perspective, consider that the almond crop in California alone requires the pollination efforts of 1.3 million colonies!

No single factor has been identified as being the causal agent for this disorder. There are three major theories being investigated by researchers. One theory is that there has been a build up in the level of pesticides in bee colonies that has reached a fatal level in many hives. A second theory is that a new parasite or pathogen is to blame. One possible organism being studied is a microbe called *Nosema* which lives in the bee's gut. The third theory is that viruses may be the cause of the problem. The most likely cause is a combination of stressors that have weakened colonies which has led to the collapse. Stress tends to negatively affect a bees immune system and the general social structure of the colony. Before the colony collapse disorder appeared, honeybee colonies were already under stress from varroa and tracheal mites. The varroa mite is a blood-feeding parasite that transmits viruses. The tracheal mite itself causes little negative consequences for a bee hive, but it may exacerbate weakness in already weakened colonies.

It is interesting that a similar phenomenon of disappearing hives was documented in the 1880s, 1920s, and the 1960s. Whether or not it was the same ailment is not known.

AFRICANIZED HONEYBEES Despite the often dramatic presentations concerning "Africanized honeybees" by the media, this group of honeybees for the most part have not and do not cause permanent injury to the general population (Delaplane, 2006). Certainly attacks which have resulted in death to

humans and livestock have occurred, hence the name "killer bees" but these attacks are rare.

The European honeybee is not well suited for life in the tropics. In an attempt to improve honey production in the tropical environment of South America, honeybees were imported from Africa to Brazil in 1956 by researchers. These bees did well in the tropics and began to hybridize with colonies of European honeybees. The "Africanized" honeybee was born. The bees slowly spread north through Central America and into North America by the 1990s.

Africanized honeybees are extremely defensive and large groups of them have been known to attack humans and livestock with very little provocation. They have also been known to take over European honeybee hives and kill the queen.

A major difference between Africanized and European bees is their permanency. European honeybees gather large amounts of honey and are well adapted for overwintering in a permanent location. Africanized honeybees do not gather large quantities of honey and respond to food shortages by migrating or swarming.

Generally Africanized honeybees do not overwinter in cold temperate climates beyond 34° latitude. The region between 32° and 34° latitude is considered the hybrid zone for African and European honeybees. Below 32° latitude, African honeybees thrive.

The venom of an Africanized bee sting is less toxic than that of a European honeybee sting! It's the number of stings inflicted during an attack that matters. When aggravated to the point of attack, the average European honeybee colony will inflict a dozen or so stings to the victim compared to hundreds of stings from a disturbed Africanized honeybee colony. The average person can tolerate 15–25 stings without medical attention. However, those with known or possible allergies should seek medical attention immediately regardless of the number of stings sustained. Anyone stung by more than 25 bees should seek attention as responses to the bee venom may be delayed in some individuals.

Banning beekeeping in your municipality is not a good idea and is not a defense against Africanized honeybees. By removing European honeybees from a region, you are actually making it easier for the nomadic Africanized bee to move in. Local beekeepers should be encouraged since they can maintain strong, healthy European colonies that would dilute any possible contamination by Africanized honeybees.

ALTERNATIVES TO HONEYBEES An excellent reference for studying alternatives to honeybee pollinators is *Managing Alternative Pollinators: a Handbook for Beekeepers, Growers, and Conservationists* (Mader *et al.*, 2010), which is available for download at www.sare.org or www.nraes.org.

BUMBLEBEES Bumblebees (*Bombus* spp.) have become increasingly popular as pollinators. There are more than 250 species of bumblebees worldwide. They are social and relatively docile. Mated females overwinter in solitary hibernation and emerge in the spring. They immediately seek a nesting site, and build a wax cell filled with pollen and nectar and lay several eggs.

As with the honeybee, a queen bumblebee mates and stores sperm in her spermatheca, located in the queen's vagina. Most bumblebee queens mate only once. When she lays eggs, she decides whether or not to fertilize an egg. Fertilized eggs develop into female workers and unfertilized eggs develop into males. Most of the eggs she lays are fertilized and become female workers. In order for a fertilized egg to become a queen, it must be fed copious amounts of pollen, and this usually only occurs towards the end of the colony life cycle.

Once the workers emerge, the queen's sole purpose is to lay eggs. The workers construct the hive and fill cells with pollen and nectar. Bumblebees collect nectar, but only enough to last for several days of inclement weather. They do not transform nectar into honey. Towards the end of the summer, female larvae are fed enough to produce queens and the reigning queen lays unfertilized eggs to develop into males. The only function of male bumblebees it to mate with the queens. After mating, the males and worker females die. The mated queens go into solitary hibernation only to start the cycle all over again the following spring.

Commercially available hives of *Bombus impatiens* generally have around 50–300 bees, which include a queen, workers, and a brood which consists of pupae, eggs and larvae. Sugar solutions are often supplied with the hive when hives are used for pollination of nectarless crops such as tomato. Different hive sizes are available depending on the size of the crop to be pollinated.

Bumblebees are not as easy as honeybees to manage, but they are extremely efficient as pollinators. They have long tongues to gather nectar from flowers whose anatomy makes nectar gathering by honeybees

difficult. In anatomically difficult flowers with long petal tubes, bees with short tongues unable to reach the nectar will often bite a hole in the base of the flower to reach the nectar. This bypasses pollination by the bee altogether.

Temperature greatly affects pollinator activity. Honeybees are not very active below 10°C, while bumblebees are active down to 7°C. While this is a very small difference, it can be important for crops that bloom when it is cool (e.g. raspberries, *Rubus* spp.) or that release pollen early in the day when it tends to be cooler (e.g. squash, *Cucurbita* spp., and cucumbers, *Cucumis sativus*).

Some flowers release pollen through pores in the anthers. In order for the pollen to be released, the anther must be vigorously shaken. A bumblebee can pollinate by grabbing an anther with its mandibles and shaking vigorously to release pollen. There is an audible buzz that emanates from the bee's throat, thus the term 'buzz pollination' was adopted for this process. Crops that benefit from buzz pollination of bumblebees include tomatoes (*Solanum lycopersicum*), peppers (*Capsicum annuum*) and blueberries (*Vaccinium* spp.). Bumblebees are also more docile than honeybees, thus they are good for greenhouse use.

Carpenter bees (*Xylocopa* spp.) are often confused with bumblebees. They look like large black bumblebees without pollen baskets on their hind legs. They are solitary and have not been of much use in pollination enhancement since they tend to cut holes in the base of corollas to access nectar, thus they don't distribute pollen very effectively.

OSMIA BEES Osmia bees (often called mason bees, blue orchard bees, and hornfaced bee) are solitary bees that are extremely important in crop pollination. While current interest in the mason bee began to increase sharply in the 1990s, mason bees (*Osmia cornifrons*) have been used for apple (*Malus domestica*) pollination since the 1940s. While there are dozens of species in the genus *Osmia*, only three species (*Osmia lignaria, Osmia californica*, and *Osmia cornifrons*) are currently commercially available for pollination.

Osmia bees are solitary, there is no hive or colony and each bee is an independent entity. The female constructs her nest, gathers pollen and lays eggs on her own with no help from other bees. Even though they are independent, many Osmia bees are gregarious, meaning that they build their nests next to other Osmia bee nests. This makes it easier to commercially raise Osmia bees as large numbers of nests can be cultivated in a single structure.

The females are the pollinators; males may gather a little nectar from flowers, but they really only live to mate. Once they mate, they die. Males are smaller than females and both are generally metallic blue, black or green in color. Females have pollen-collecting hairs on their abdomen as well as a pair of horn-like appendages on their faces. Males do not have facial horns, but often have a tuft of white hair in the center of their face. Even though they have a stinger, females rarely sting and if they do, the sting is not very painful.

Most Osmia bees are univoltine, meaning that there is only one generation per year. All *Osmia* species are only active in the spring, with the exception of *O. californica*. Adults emerge from eggs laid the previous spring and are active for only a few weeks. During this time they mate, build their nests, lay eggs and die. Larvae develop into adults over the summer and lie dormant over the winter. Since they are only active early in the spring, they are effective pollinators for a limited, yet important, number of crops. They are extremely important in fruit crop pollination.

Managed Osmia bees build nests in tunnels lined with cardboard in holes drilled in wooden blocks. The tubes are lined with a type of cement (hence the name mason bees) made up of mud gathered from nearby areas by females as they prepare to lay their eggs. The females construct one cell at a time, fill it with pollen and nectar, lay an egg, then cap the cell with more mud. Each nest is finished with one empty cell with a thick end wall. The empty cell provides extra protection from predators and wasps who might try to lay eggs in the nest. Each female normally lives for about 1 month and builds one to six tunnels, each with five to 15 eggs in each.

There are two options to choose from when considering using mason bees as supplemental pollinators. Mason bees can be 'trap nested' from wild populations or purchased from commercial sources. Trap nesting ensures that the bees are those native to your habitat. Purchased bees may or may not be native to your area.

ALKALI BEES (*NOMIA MELANDERI *COCKERELL) Alkali bees are an effective pollinator of alfalfa in the western USA. The alkali bee is a gregarious bee almost as large as a honeybee which nests in the soil. They are black with copper-green stripes across

the abdomen and the males have much larger antennae than the females. They generally build up to 100,000 nests over a 23 m^2 area. Their nests, each with 15–20 cells, are generally 1 cm wide and extend 7–13 cm vertically into the soil. Each cell is oval and slightly larger than the main nesting tunnel and is about 1 cm long. It is lined with soil followed by a waterproof liquid produced by the bee. A pollen ball made up of eight to ten bee loads of pollen mixed with nectar is placed in each cell.

Adults emerge from their cell after spending 10 months as a dormant, fully grown larva from late June to late July. Males emerge several days before the females. A female lays 15–20 eggs over the course of her active, 1 month-long life as an adult. Males mate with the female during the first day of hive construction and each male mates with multiple females. On average each female lays one egg/day.

The primary food source for alkali bees is alfalfa. Females seek nectar and pollen while males seek nectar only. Most flowers are pollinated by female bees as pollen is generally only released when females are searching for pollen.

Artificially prepared nesting sites or beds can be developed in regions where the alkali bee is not normally found and the reader is advised to examine the book by McGregor (1976) if interested. Construction of alkali bee beds may be more cost effective than honeybee hive rental. However, the utility of alkali bees for pollination is somewhat limiting as: (i) beds can only be used in areas with limited rainfall during bloom; (ii) beds must be constructed where the crop is to be grown; and (iii) they must be constructed months before the crop is planted. In addition, an alkali bee bed can be lost to pests with little or no warning.

ALFALFA LEAFCUTTER BEES (MEGACHILE PACIFICA PANZER) The alfalfa leafcutter bee gets its name from the fact that it lines its above-ground nests, that are hollow tubes or tiny holes, with circular sections of alfalfa (*Medicago sativa*) leaves, and on occasion, petals of ornamental flowers.

The alfalfa leafcutter bee is a gregarious solitary bee slightly larger than a housefly. The female begins constructing nests in hollow tunnels in which she barely fits, as soon as she emerges in the spring. Each cell in the nest is lined with leaf sections cut from alfalfa, then filled with pollen and nectar. Pollen and nectar are gathered from mostly alfalfa, clovers (*Melilotus* spp., *Trifolium repens* L.), and mint (*Mentha* spp.). The female lays her egg, caps off the cell with leaf sections, and begins construction of the next cell in the nest. This process continues for about 2 months as long as pollen and nectar are available. The average female mates only once and lays about 30–40 eggs, of which two or three of the resultant adults will be male. The male generally seeks nectar only and is inefficient in pollinating crops. The female seeks pollen and nectar and may visit up to 15 flowers/min, thus she is a very effective pollinator.

Development from egg to adult is extremely temperature dependent. It takes 15 days for eggs to hatch at 16°C and 2 or 3 days at 35°C. Development through four instars to the pre-pupal stage takes 11 and 35 days from egg hatch at 16°C and 35°C, respectively. Many larvae can be lost during the first two instars if the temperature is above 26°C or too cold for feeding. If larvae die during their development, an empty, pollen-filled celled called a 'pollen ball' is all that remains.

In cooler climates, development stops at the pre-pupal stage and the bee will overwinter in a dormant state. As temperatures warm in the spring, the pre-pupae molt into pupae. After about 1 week, adults emerge. In warmer climates, development may continue past the pre-pupal stage without dormancy and a second generation of bees may emerge. One major problem with second-generation bees is a high incidence of chalkbrood mortality, a disease of leafcutter bees. When second-generation bees emerge from the nest, they chew through chalkbrood contamination from the previous generation and may become covered with spores in the process. The second-generation adults then return to the nest and contaminate any new nests they build.

Males emerge before females, and once females emerge, mating begins. Females mate once while males may mate several times. Sperm is stored in a spermatotheca and the female will begin to lay eggs a 1 or 2 days after mating.

Alfalfa flowers are like most other legume flowers in that they have a unique design which is important in pollination strategies. A legume flower normally has one large 'normal' petal with two 'wing' petals, one on each side of the 'normal' petal. Two other petals on the bottom of the flower are fused together to form the 'keel' petal which encloses the stamen under tension. When the fused keel petals are slightly separated by a pollen-seeking insect, the stamen is released rapidly, smacking the

'normal' petal. Once the stamen has been released from the keel, it does not return to its former position, but remains exposed. The flower is then said to have been tripped. During the tripping process, the insect is dusted with pollen. Insects are often hit on the head during tripping. Leafcutter bees are much less annoyed by the process compared with honeybees, as honeybees will seek other sources of pollen and nectar rather than get smacked on the head.

Alfalfa leafcutter bee nesting is easily encouraged using commercially available nesting boards. Larvae-filled nesting boards are stored in a cool, dry place between –1°C and 4°C. They are removed from storage the following spring as pollinators are desired.

STINGLESS WILD BEES Two important genera of stingless social bees are *Melipona* and *Trigona*. These bees are particularly important in crop pollination in Mexico, Central and South America, Africa, Southern Asia, and Australia. Besides their importance in pollination, they also produce honey and wax.

Even though these bees are stingless, some do have mandibles strong enough to inflict a mild bite and some emit a substance from their mouth that is irritating to the skin. In general, these bees do not injure humans working with or around them.

The honey produced by stingless bees is quite variable in quality. The wax secreted by stingless bees is mixed with propolis, thereby causing the wax to be black. It is used for waterproofing and ink.

These social bees have queens who are attended to by worker bees. Each nest has a single egg-laying queen and up to 50 virgins.

COMMERCIALLY AVAILABLE POLLINATORS Many horticultural crops benefit from the introduction of extra pollinators into the production field due to a lack of natural pollinators. While most of the pollinators available for either rental or purchase are expensive, the benefit of their use is quickly realized by increased yield.

There are many factors which determine how many pollinators you might need for a specific crop at a specific location. These factors include: (i) crop; (ii) size of planting; (iii) time of year; (iv) wild pollinator populations; and (v) what commercial pollinator you plan to use. As a very general rule of thumb, you need at least two to five honeybee hives, ten bumblebee hives, 50,000 leafcutter bees or 620 female mason bees per hectare.

HONEYBEES Probably the most widely used accessory pollinator is the honeybee. Hives are available for rental, and demand is usually high. Thus it is important to set up rental agreements early to ensure that you will be able to get the hives you need. Make sure that you only sign a rental and liability agreement that you are comfortable with and seek professional guidance if you are unsure about the agreement.

There are a number of factors which should be considered when entering a hive rental agreement. All parties involved in the transaction must be clearly identified. This includes the grower, the beekeeper, and the transportation specialist. The crop which will be pollinated and its location should also be included as well as a glossary of terms which may not be familiar to all parties. The number and size (or strength) of hives, the rental price, and the length of the rental must be included, as well as any transportation or maintenance fees. An explicit listing of pesticide practices utilized by the grower is often useful in preventing any miscommunications regarding appropriate exposure of hives to pest management techniques. This list should include pest management practices utilized prior to the hive rental. Make sure that both parties agree on removal of hives after the pollination season, so that they don't interfere with ongoing farm practices, especially pesticide application. Liability for injuries caused by bees should be agreed upon. This is especially true if visitors will be on farm during the pollination season.

BUMBLEBEES Bumblebee (*Bombus terrestris* and *Bombus impatiens*) hives are purchased rather than rented (Fig. 5.6). A colony with a queen along with workers and a brood (pupae, eggs, and larvae) are supplied in a plastic and cardboard hive along with a sugar solution for supplemental feeding of the hive. Different hive types are available with different pollination capacities. Bumblebees are most often used in protected culture of fruits and vegetables. They are not as aggressive as honeybees and a hive of 50 or so bumblebees can do the work of 20,000 honeybees.

It is important that the hive be adequately shaded in the greenhouse. Bumblebees work best at a temperature range of 10–30°C. Air vents must be screened to prevent the escape of bees from the

Fig. 5.6. A commercial bumblebee hive often used for pollinating greenhouse crops such as raspberries (*Rubus* spp.) or strawberries (*Fragaria* × *ananassa*).

greenhouse. The hive should be located as far away from carbon dioxide supplementation as possible.

Since bumblebees rely on UV light for navigation, it is important to manage lighting conditions of the greenhouse with the bees in mind. Their hive should be about 2 m off the floor with the hive entrance facing east. If daylengths are extended with artificial lighting, extend the light period before sunrise, not after sunset. Before sunrise the bees will be in the hive and turning lights on will stimulate them to pollinate. If you extend the light before sunset, bees are disoriented when the lights are shut off and are unable to return to the hive.

Great care must be given to the use of any pest management schemes employed in the greenhouse. It is often necessary to remove the hive(s) from the greenhouse when pesticides are used. Most commercial hives have two entrances. One allows bees to fly in and out of the hive while the other only allows flight into the hive. To remove the hive from the greenhouse, simply close the two-way entrance. After about 1 h, all bees will have returned to the hive. It can then be removed from the greenhouse until it is safe for the bees to return to flight. Make sure the hive is protected from ants, since ants are highly attracted to the sugar solution feeding the bees. Larger hives are available for field or high tunnel use.

GREENBOTTLE FLIES (LUCILIA CAESAR) The greenbottle fly is available for pollination enhancement that is often needed in the production of certain seed crops. Sometimes both male and female lines in field hybridization produce little pollen or nectar, and are thus not attractive to "normal" pollinators. Such crops include cauliflower (*Brassica oleracea* Botrytis Group), cabbage (*Brassica oleracea* Capitata Group), rapeseed (*Brassica napus*), lettuce (*Lactuca sativa*), endive (*Cichorium endivia*), radicchio (*Cichorium intybus*), carrot (*Daucus carota*), onion (*Allium cepa*), leek (*Allium ampeloprasum* var. *porrum*) and asparagus (*Asparagus officinalis*). The adults visit flowers searching for nectar and in the process distribute pollen on their bodies. In order to be effective pollinators, their bodies must come into contact with anthers when searching for nectar. This is totally dependent on flower structure. The pollen is carried to the next flower they visit.

Greenbottles are purchased as pupae mixed with sawdust. Approximately 1 kg of this mixture is evenly dispersed over 100 m^2 area once a week during pollination. It should be dispersed under the shade of the canopy in the morning or evening and protected from mice. The pupae–sawdust mixture has a shelf life of only 1 week and should be stored at 6°C in the dark. Flies emerge in about 3–4 days at 24°C.

ORCHARD MASON BEES (OSMIA SPP.)/ALFALFA LEAFCUTTER BEES Many commercial sources of Osmia bees and alfalfa leafcutter bees exist. Bees may be purchased in a number of forms. Which you choose is up to you but it is imperative that you become educated in handling the dormant bees before embarking on their use. For specific information see the before-mentioned reference (Mader *et al.*, 2010) on alternative pollinators, as a discussion of their handling is beyond the scope of this text.

Fertilization

The final phase of floral development occurs with fertilization. Fertilization ultimately results in the formation of a seed or seeds which then influence the growth and development of the fruit. Fruit growth and development are really extensions of floral development; however, we separate the two for clarity.

DOUBLE FERTILIZATION Fertilization in most plants is a two component event called double fertilization. In one phase of fertilization, one of the sperm cells from the pollen grain unites with the egg cell to form a single-celled zygote. The zygote undergoes mitosis and transforms into a multi-celled embryo. The second sperm cell from the pollen grain fuses with the polar nuclei (remember it's a diploid entity

of the female gametophyte) to form the triploid endosperm. The endosperm is parenchymatic tissue with the primary function of food storage. In some seeds it is absorbed into large fleshy seed leaves (e.g. green beans, *P. vulgaris*) while in other seeds, it remains separate as a starchy entity (corn, *Zea mays*). Together the embryo and the endosperm form the bulk of a seed. The seed or seeds formed from the fertilization of a flower's egg(s) have a profound influence on the development of the fruit.

Fruit

There is often confusion about whether certain horticultural commodities are a fruit or a vegetable. It depends on whether you are speaking botanically or from a horticultural perspective. Botanically, a fruit is a ripened ovary along with any harvested accessory tissues. The horticulturist would probably refine that definition to limit fruits to a commodity used for dessert or treats compared with those employed in the main meal. For example, a green bean is a fruit to a botanist while a horticulturalist would place it in the vegetable category. Unfortunately, sometimes the classification is for political reasons, such as the United States Department of Agriculture (USDA) classification of the tomato as a vegetable so that ketchup served with French fried potatoes can be considered a vegetable in school lunch programs.

Fruit set

The first stage of fruit growth and development is called fruit set. Fruit set describes a visible stage of development where it is apparent on inspection of the remnants of the flower that a harvestable fruit is beginning to develop. It is a point in development where a physiological decision is made to either abort the embryo and ovary or to go ahead with fruit development. Fruit set is most often quantified as a percentage of flowers present at bloom which appear to be developing into a harvestable fruit. Most fruit and vegetable crops require both pollination and fertilization for fruit set and growth.

Seedless fruit/parthenocarpy

Seedless fruit production is known as parthenocarpy. There are three different forms of parthenocarpy: (i) vegetative parthenocarpy; (ii) stimulative parthenocarpy; and (iii) stenospermocarpy.

Vegetative parthenocarpy

Fruit set without pollination or fertilization is called vegetative parthenocarpy. Seedless cucumber (*C. sativus*), pineapple (*Ananas comosus*), 'Satsuma' mandarin (*Citrus reticulata*), 'Washington' navel orange (*Citrus sinensis*), some sycomore figs (*Ficus sycomorus*) and dessert banana (*Musa acuminata*) are examples of fruits that form by vegetative parthenocarpy.

Stimulative parthenocarpy

Fruit set with pollination but without fertilization is called stimulative parthenocarpy. Seedless clementines (*Citrus reticulata*), eggplant (*Solanum melongena*) and 'Black Corinth' grape (*Vitus vinifera*) are examples of fruit formed via stimulative parthenocarpy. Stimulative parthenocarpy can also be achieved via stimulation with plant growth regulators or other stimuli, such as wasps as is the case in some figs (*F. sycomorus*).

Stenospermocarpy

Fruit production when pollination and fertilization are quickly followed by embryo abortion is called stenospermocarpy. Seedless grapes (*Vitis* spp.) such as 'Thompson Seedless' (*Vitis vinifera* 'Thompson Seedless') result from stenospermocarpy. Fruits without seeds tend to be smaller than those with seeds. This is because seeds are a rich source of the plant hormone, gibberellic acid (GA). Many seedless grape cultivars are sprayed with GA after bloom to increase size. (GA is also used during bloom to reduce the number of berries set in each grape cluster.)

In some horticultural crops, parthenocarpy is controlled through controlled crosses. This is the case with seedless watermelon (*Citrullus lanatus* var. *lanatus*). This is a good example of controlled stimulative parthenocarpy, since only pollination is required for fruit growth and development. The pollinated cultivar does not produce viable eggs, thus fertilization is impossible (Fig. 5.7). The pollinated cultivar is obtained by crossing an inbred tetraploid female with an inbred diploid male parent to produce triploid seed. (The reciprocal cross of diploid female parent with tetraploid male parent does not produce seed.) Tetraploid plants produce only 5–10% the amount of open-pollinated cultivars, thus triploid seed is very expensive. Plants from triploid seeds (33 chromosomes) are sterile

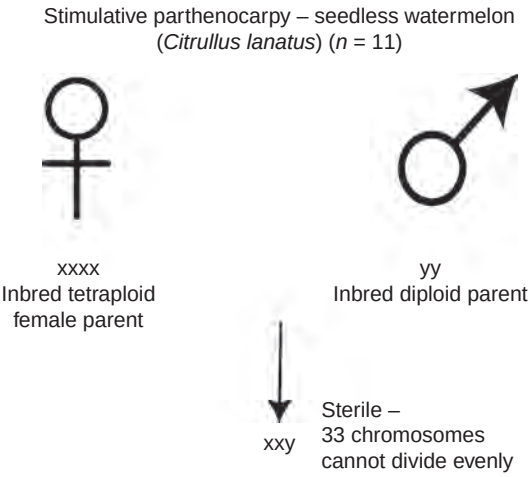

Fig. 5.7. Genetic control of seedlessness in watermelon (*Citrullus lanatus*). (Gender symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science, ian.umces.edu/symbols/.)

since their chromosomes cannot be divided equally during meiosis. However, parthenocarpic fruit will develop if a diploid pollinizer is planted in with the triploid cultivar.

The fruit-set signal

Unless the species is vegetatively parthenocarpic, the most important factor determining the amount of fruit set is pollination. The act of pollination sends a signal to the ovary to continue development. Growing pollen produces GA which enhances auxin production in the style and ovary. GA is the primary signal which induces the secondary signal, auxin. The flower abscisses if this gibberellin signal is not present. In some species, pollination can be replaced with an application of GA, and GA or auxin can also be effective in enhancing fruit set in some species in years when pollination is poor.

Seed set

While pollination is all that is needed for fruit growth to continue in some species, in most species fertilization must occur for the fruit to continue developing. In these crops seeds are needed for fruit growth. Assuming effective pollination occurs, temperature will probably determine whether or not fertilization takes place. The pollen tube must grow down the style quickly enough so that the sperm cells can effect fertilization before the ovule dies. The temperature that is best for pollen growth varies with species.

Seed set occurs once fertilization of the egg has taken place and the chance of embryo abortion has diminished. While one might argue that seed set and fertilization are one and the same, the chance of embryo abortion immediately after fertilization distinguishes the two events. Seed set is characterized by a sharp increase in the production of gibberellins, auxins and cytokinins, each playing an important role in further fruit and seed growth.

Fruit drop/fruit thinning

In all cases, once fruit growth begins, adequate photosynthates must be translocated to the developing fruit. If there are too many fruit developing on a plant, the available photosynthate will often be translocated to the larger, stronger fruit, resulting in the abortion of the embryo and abscission of the weaker fruit from the plant. In tree-fruit production, this is called 'June drop'. One of the tasks of a fruit grower is to prevent excessive fruit set while at the same time promoting sufficient set for a full crop.

Fruit growth

After fruit and/or seed set, fruit growth results from both cell division and expansion. In most crops, an initial period of rapid cell division occurs in all parts of the fruit and is dependent on growth regulators produced by the developing seeds. Fruit usually do not appear to grow very much during this stage even though the number of cells in it is increasing dramatically. Cell division in many crops seems to be regulated by hormones, particularly gibberellins, produced by the developing seeds. Final fruit size is often highly correlated with the number of seeds developing in the fruit. When one or more seeds in a multi-seeded fruit abort, lopsided and misshapen fruit result. Parthenocarpic fruits don't have seeds and how their growth is regulated is not well understood.

Fruit gradually shift from cell division into a period of cell expansion. Some tissues within the fruit continue division while other tissues begin cell expansion. In general the tissues that make up

the bulk of the mature fruit undergo greater cell expansion, while the other tissues are likely to continue cell division for any increase in size. By far, cell expansion accounts for the greatest increase in fruit size.

Fruit ripening

An in-depth look at maturity and ripening is presented in Chapter 15, this volume. We briefly address both here for completeness. Fruit are considered mature if they will ripen once removed from the parent plant. Fruit ripening is the process of becoming edible after maturity has been reached. Crops can be harvested either: (i) mature but not ripe; or (ii) mature ripe. Strawberries will not continue to ripen once harvested, thus they are harvested mature ripe. Pears are not edible when harvested and must ripen before being consumed.

In general ripening coincides with embryo maturation. We often limit our discussion of ripening to the fleshy fruited commodities. Fleshy fruit are either climacteric or non-climacteric, a quality very important in postharvest considerations. Climacteric fruits have a rapid concomitant increase in respiration and ethylene production. This increase occurs just prior to ripening and is quickly followed by fruit senescence. Climacteric fruit include tomatoes (*S. lycopersicum*), bananas (*Musa* spp.) and peaches (*P. persica*). Non-climacteric fruit such as grapes (*Vitis* spp.) and strawberries (*Fragaria* × *ananassa*) do not exhibit this phenomenon. Ethylene is the hormone regulating ripening and senescence, but exposure to ethylene does not automatically induce ripening. A fruit must be 'ready to ripen' before ethylene can exert its effect. At that point they are called mature green. Ethylene production by plant tissues is autocatalytic, meaning that ethylene induces ethylene production.

Many changes to fruit tissues occur during ripening and they will be discussed in detail in Chapter 15, this volume. Generally speaking, color changes, sugars increase, acids decrease and tissue softening takes place. Ultimately fruit quality will deteriorate as the fruit begins to senesce after ripening.

Seeds

A seed, consisting of an embryo, the endosperm and a testa (seed coat) develops from an ovule in an ovary. The embryo is the newly developing plant inside the seed. In order to truly appreciate how marvelous its development is, one must step back and review the situation existing inside the ovary just prior to fertilization. The multi-cellular embryo sac resides inside the ovary and has a polar organization from one end to the other. The two ends of the embryo sac are the micropylar end and the chalazal end. The egg cell and its synergid cells are close to the micropylar end while the antipodal cells are close to the chalazal end. A central cell with two nuclei is located about midway between the two ends. The pollen tube grows through the micropyle opening and one of the sperm cells traveling down the pollen tube fertilizes the egg cell to form a one-celled diploid zygote. The other sperm cell combines with the two polar nuclei of the central cell to form the triploid endosperm. The endosperm serves as a nutrient reservoir for the embryo and may become a separate entity or it may be absorbed into the embryo during seed development.

Embryo development – morphogenesis

Embryo development is often divided into three overlapping stages: (i) morphogenesis; (ii) maturation; and (iii) desiccation (Evert and Eichorn, 2006). During morphogenesis, the polarity first observed in the embryo sac is preserved in the typical plant body which consists of a basal root apex, a distal shoot apex and connecting tissue between the two (de Smet *et al*., 2010). The polar nature of development in the embryo seems to be controlled maternally and determined in the embryo sac. However, since somatic cells of many plants can be forced to undergo embryogenesis, other regulators of polarity must exist.

The single-celled zygote undergoes an uneven transverse (divides along the equator of the cell) cell division resulting in a smaller apical cell which ultimately forms the embryo and a larger basal cell which develops into the suspensor and hypophysis. The suspensor connects the embryo with maternal tissue while the hypophysis gives rise to the root cortex and cap.

An embryo's polarity defines its axis of development. Precisely regulated cell division leads to further development in a series of events that partition the embryo into three distinct zones: (i) an apical zone which includes the cotyledons, the shoot apex and the upper part of the hypocotyl; (ii) a mid-zone which includes most of the hypocotyl; and (iii) a basal zone which includes the root apex. These zones

have been observed in many species during embryogenesis. The regulation of the development of these zones has been shown to be under precise genetic control in studies with *Arabidopsis* mutants and auxin appears to be the primary phytohormone involved in embryo developmental patterns (de Smet *et al*., 2010).

There may be a role for cytokinins for primary root development (Müller and Sheen, 2008) and brassinosteroids may take part in embryo development (Chandler *et al*., 2009; Scacchi *et al*., 2009). Gibberellins and abscisic acid (ABA) are mainly involved in the later stages of embryo development, particularly ABA maintenance of dormancy and gibberellin promotion of germination (Holdsworth *et al*., 2008).

The shoot, cotyledons and hypocotyl are all derived from the apical cell which formed after the first division of the fertilized egg. Root development is a little more complicated. The top cell of the suspensor, which was derived from the basal cell after the first division of the fertilized egg, forms the hypophysis which develops into the root cap, root cap initials and ground meristem initials. The remainder of the root apex develops from the apical cell.

The first two embryonic organ systems to develop are the cotyledon and the axis, which form in response to polar auxin transport. Their formation begins in the globular stage of embryo development but they are not visible until the heart stage. We know that differentiation begins in the globular stage since embryos treated with auxin transport inhibitors in this stage, or mutants deficient in auxin transport develop deformed cotyledons. Separate cotyledons do not form, but rather, a ring of cotyledon-like tissue develops as a ring around the apex. Another important step in embryo development is the development of specific tissues within the organs, specifically the protoderm, the ground tissue and the procambium. These tissues become apparent in the transition from the globular to the heart stage.

The embryo eventually moves into a stage where lipid, protein and carbohydrate reserves are developed in all cells. These reserves are especially important in the cotyledons of species which do not develop distinct food reserves in an endosperm. These reserves serve as a nutrient source for developing seedlings until the seedling can become photosynthetic. The embryo finally enters a period of desiccation.

Endosperm development

The endosperm results from the union of the polar nuclei with a sperm cell from the pollen grain (Evert and Eichorn, 2006; Dumas and Rogowsky, 2008). In some species it remains a separate entity from the embryo, but in others it is absorbed by the cotyledons. The endosperm has two very important roles in seed development. One is to be a source of nutrition for the developing embryo and the other is to at least partially direct development of the seed (Berger *et al*., 2006).

In angiosperms there are two types of endosperm: (i) nuclear; and (ii) cellular. The cellular type of endosperm is limited to the lower or basal angiosperms. Its development is similar to that of the embryo, which suggests that it may have evolved as some sort of simple auxiliary embryo.

The nuclear type of endosperm is the most common type of endosperm in angiosperms. Nuclear endosperms have a series of nuclear divisions followed by polar migration of the nuclei forming sequestered units or domains, much like in the developing embryo. The cell-like structure of the nuclear endosperm is achieved with the formation of alveoli, open-ended tube-like structures. Alveoli are found only in nuclear-type endosperm and in the female gametophyte of gymnosperms. Gymnosperms do not have endosperm, but rather the female gametophyte enlarges considerably while storing nutrients during embryo development.

Though endosperms vary considerably among angiosperm species, they all have common attributes. These include: (i) a mother–offspring interface; (ii) an embryo–endosperm interface; (iii) an epidermis; and in some (iv) a separate storage tissue.

The endosperm is designed to transfer nutrients from the mother plant to the developing embryo. In both monocots and eudicots (most but not all dicots) most nutrients, mainly sucrose, is absorbed by the embryo through the mother–offspring interface by means of specialized haustoria, or food-absorbing outgrowths, in the chalazal area of the ovule (the end away from the micropyle). This area of the endosperm is often called the chalazal endosperm. The area right next to the embryo is called the micropylar endosperm. It is important for regulating development of the embryo by regulating sugar uptake. It also protects the embryo with anti-fungal proteins.

Endosperms accumulate certain compounds as food reserves for the embryo. Starch is the main

storage compound in monocots and it is stored in a mass of enlarged cells called the starchy endosperm. Most other angiosperms store lipids and proteins in their cotyledons rather than a large separate region as in the monocots.

All endosperm possess an epidermis called the aleurone layer. It contains proteins which prevent precocious germination and promote desiccation tolerance. Enzymes are released from the aleurone layer during imbibition that mobilize stored nutrients for use by the rapidly growing embryo. The aleurone layer is present even in species where most of the endosperm is absorbed by the embryo, indicating a role in seed maturation and germination.

The endosperm is triploid, two sets of chromosomes are maternal and one is paternal. In order for normal development to occur, this two-to-one balance must be maintained. This suggests that a large portion of endosperm development is maternally controlled. When the balance is switched via novel breeding and molecular techniques, endosperm development is abnormal.

Seed coat development

The testa originates from the integuments of the ovule and serve a protective function surrounding the embryo/endosperm complex. The seed coat often has adaptations for improved dispersal such as hook-like hairs or glue-like substances that allow seeds to become attached to animals for deposition away from the parent plant.

Maturation and entrance into dormancy

As the embryo reaches a stage of maturation, most cell division has been completed. Further growth is through cell expansion and an embryo may grow 100-fold during that time. During the cell expansion period a large accumulation of storage compounds (starches, lipids, and proteins) occurs. Many of these storage compounds are harvested by humans for food. From a seed's perspective, storage compounds are an important energy source for germination.

Another important physiological process that occurs during seed maturation is the development of desiccation tolerance by the seed, the embryo in particular. There is a group of proteins called deyhdrins which develop as the seed matures. These proteins seem to sequester ions to prevent crystallization during desiccation. Dehydrins may also form a protective layer surrounding various membranes in embryonic cells.

Most seeds enter a period of dormancy after maturation which can range from several days to years. In addition, some seeds require exposure to cool moist conditions before they will germinate. This exposure is called stratification, and will be discussed in Chapter 9, this volume. Seeds of some species do not go through a maturation stage per se but rather may germinate immediately upon release from the fruit. This type of seed is called viviparous.

Seed dormancy

Definitions of dormancy are difficult to develop because dormancy can only be measured by the absence of germination. Even so, seed dormancy has been organized into five major categories: (i) morphological; (ii) physical; (iii) physiological; (iv) morphophysiological; and (v) combinational dormancy (Baskin and Baskin, 2004). It is also often characterized as primary or secondary dormancy. Generally speaking, a seed is dormant if it cannot germinate in a specified amount of time under any combination of physical environmental factors that are otherwise favorable for its germination once it is non-dormant (Baskin and Baskin, 2004).

Morphological dormancy

Morphological dormancy is characterized by an incompletely developed embryo at seed harvest. Once the embryo grows to maturity it will readily germinate. Celery (*Apium graveolens*) is an example of seed with morphological dormancy.

Physical dormancy

Physical dormancy is caused by some characteristic of the seed which prevents it from imbibing water and germinating under normally favorable conditions. Inability to imbibe water may be caused by a layer of waterproof cells in the seed coat. Imbibition can only take place once this barrier is broken down by fluctuating temperatures, repeated freezing and thawing, fire or passage through an animal's digestive tract.

Another characteristic which prevents water imbibition is the presence of a specialized structure at the hilum, the area where seed was attached to the ovary via a placenta. One such structure is

called a lens and occurs in legumes in the family *Fabaceae*. Only when cells in the lens are disrupted can the seed imbibe water. Another structure called the chalazal plug or cap control water imbibition in some species in the *Bixaceae, Cistaceae*, and *Malvaceae*.

Physiological dormancy

Physiological dormancy is the most widespread type of seed dormancy and is often subdivided into three further levels: (i) deep; (ii) intermediate; and (iii) non-deep. In order to determine the type of physiological dormancy of seeds in a particular species, embryos are excised from the seeds and placed under conditions known to favor germination in that species. Seeds with deep physiological dormancy require 3–4 months of warm (>15°C) or cold (0–10°C) stratification in order for their embryos to germinate and treatment with GA does not hasten germination. Some examples include *Acer platanoides* and *Leptecophylla tameiameiae*.

Intermediate physiological dormancy is characterized by a requirement for 2–3 months of cold stratification. Dry storage shortens the stratification period in some species and treatment with GA promotes germination in some species. An example is *Acer pseudoplatanus*.

Most species exhibit non-deep dormancy. Treatment with GA breaks the dormancy as can scarification, dry storage, or cold or warm stratification. Non-deep physiological dormancy is divided into five levels numbered one through to five, and most species are in type one or two. The five types differ in seed germination temperature requirements.

Morphophysiological dormancy

Seeds that exhibit morphophysiological dormancy have an underdeveloped embryo *and* some other physiological element to their dormancy, which usually requires some form of stratification. An example of a species with this form of dormancy is ash (*Fraxinus excelsior*).

Physical with physiological dormancy

Seeds with a hard seed coat and a non-deep physiological requirement to end dormancy are put into this category (e.g. *Geranium* spp.).

Requirements for breaking dormancy

Before sowing seeds of any horticultural crop, seed dormancy of that species should be well understood if success is expected. Usually specific requirements involve light, water, temperature or scarification treatment(s) to improve germination. Specific requirements are easily found in plant propagation texts, seed packets or with a quick search of the Internet.

Germination

Germination is a highly regulated process which occurs only when environmental conditions are favorable for growth of the species in question (Finch-Savage and Leubner-Metzger, 2006; Nonogaki, *et al*., 2010). Germination encompasses those events occurring from water imbibition through to radicle emergence (Bewley, 1997; Nonogaki, *et al*., 2010). It is interesting that almost all cellular and metabolic events which occur between imbibition and radicle emergence in non-dormant seeds occur in dormant seeds, yet the radicle fails to emerge from dormant seeds.

Imbibition

Water imbibition is the first step of germination (Nonogaki, *et al*., 2010). Water uptake by seeds is triphasic. There is a rapid initial uptake of water (phase I) followed by a plateau phase (phase II). A third phase (phase III) involves the rapid uptake of water with elongation of the embryonic axis which results in radicle emergence and the completion of germination. Dormant seeds can progress through phases I and II, however, dormant seeds do not enter phase III.

Structural changes

Water uptake in phase I immediately leads to structural changes in membranes. They go from a gel phase that was induced during the maturation and drying stages of the seed development to a 'normal' hydrated liquid-crystal state (Crowe, *et al*., 1989). During the transition from gel to liquid-crystal, extensive leakage of solutes and low molecular weight substances into the surrounding imbibition fluid occurs (Copeland and McDonald, 1985). Membrane repair to correct the leakage problem occurs during the hydration process, but the mechanism of repair is unknown.

Metabolic changes

There is a rapid increase in metabolic activity on imbibition (Nonogaki, *et al*., 2010). The structures and enzymes needed for cellular metabolism are present but are not necessarily functional within the dry seed. Part of the amazing physiology of a seed is the fact that it survives the intense dehydration associated with desiccation at maturity. Full metabolic status and functioning occurs within several hours after the initiation of imbibition.

One of the first metabolic changes observed during germination is a rapid increase in respiration which is detected within minutes. The rate of oxygen uptake and carbon dioxide release slowly declines after the initial surge. Radicle emergence from the seed is accompanied by another respiratory burst. Though mitochondria are poorly differentiated due to desiccation during seed development, there are enough enzymes present to produce enough ATP for several hours after imbibition (Hourmant and Pradet, 1981). Two distinct patterns of mitochondrial development occur during germination, depending on the type of food reserves in the seed. Starch-storing seeds normally repair and activate pre-existing mitochondria while oil-storing seeds typically produce new mitochondria (Morohashi, 1986). In both cases, the glycolytic and oxidative pentose phosphate pathways both resume during phase I, and the Kreb's cycle is activated.

All cellular parts needed for protein synthesis are present in mature dry embryos except for polysomes (Nonogaki, *et al*., 2010). Immediately upon imbibition, singular ribosomes gather into polysomes and start synthesizing protein. New ribosomes are also made. Initially, mRNA that is already present is used for protein synthesis, but with time, new transcripts are utilized for the protein code. The new mRNAs transcribed as germination proceeds encode proteins needed for normal growth and maintenance of the developing seedling, rather than those needed for germination. Even though many references in the literature speak of specific mRNAs responsible for food-storage mobilizing enzymes needed for germination, this remobilization actually occurs post-germination.

Radicle extension and the completion of germination

Radicle extension through the tissues surrounding the embryo signals the end of germination and the beginning of seedling growth (Nonogaki, *et al*., 2010). This extension growth may or may not be accompanied by cell division. There are two distinct phases of DNA synthesis in radicle cells after imbibition. The first phase is soon after imbibition and involves the repair of DNA damaged during the desiccation associated with maturation and subsequent imbibition. There is also synthesis of mitochondrial DNA at this stage. The second phase is DNA synthesis associated with post-germinative cell division.

Radicle extension is driven by turgor pressure and requires flexibility in cell walls of the root axis between the cap and the hypocotyl. How does turgor increase in these cells to cause radicle elongation and emergence? Osmotic potential of radicle cells somehow becomes more negative due to accumulation of solutes with concomitant uptake of water, but the mechanism for such changes is not yet known.

The radicle must go through tissues surrounding the embryo to emerge from the seed (Nonogaki, *et al*., 2007). In some species the tissue yields easily to the pressure exerted by the radical, while in other species there is considerable resistance. The resistance declines during germination due to the activity of cell-wall loosening enzymes (hydrolases).

Metabolism of dormancy maintenance and termination

Some seeds may lose their dormancy while dry and their metabolism is very low. Many, however, lose their dormant status only after imbibition. Imbibed dormant seeds have a high level of metabolic activity and they are receptive to external stimuli (light, temperature, chemical treatment) that remove dormancy and/or induce germination. The primary event in release from dormancy is the reception of the dormancy-breaking signal by the embryo followed by metabolic and hormonal changes which result in the emergence of the radicle.

One of the most well-known primary signal receptors is phytochrome. P_r is transformed to P_{fr} by red light. P_{fr} is the active form, but what this active form of phytochrome causes within the seed that leads to germination is not known.

Primary versus secondary dormancy

Freshly harvested mature seeds are in a state of primary dormancy that was induced by ABA while

the seeds were still on the mother plant (Finch-Savage and Leubner-Metzger, 2006). Secondary dormancy is often associated with unfavorable environmental conditions after primary dormancy has been removed that do not favor germination.

Seed coat and endosperm control of germination

In some seeds, the endosperm and seed coat covering the embryo may present a mechanical constraint that must be overcome by growth of the embryo and thus cause a form of dormancy called coat dormancy.

In non-endospermic seeds and in *Arabidopsis* (which has one-cell layer of endosperm), the seed coat alone is responsible for coat dormancy. Completion of germination in seeds with coat dormancy requires embryo growth to overcome the mechanical constraint as well as a decrease in resistance of the seed coat. In endospermic seeds both the testa and the endosperm layers have to be considered in evaluating coat dormancy.

Endosperm rupture is the germination-limiting process in seeds of members of the *Asteraceae* (lettuce), *Solanaceae* (tomato and tobacco), and *Rubiaceae* (coffee). In these species, weakening of the endosperm surrounding the radicle tip is required for radicle protrusion.

Hormonal regulation of seed dormancy

One of the most intriguing questions in horticultural physiology cannot be easily answered. How does an embryo emerge from a seed to complete germination, and how is emergence blocked so that seeds can be maintained in the dormant state? The seed is the beginning of the next independent generation of any plant species and dormancy is the intrinsic block to its germination into a new seedling.

We can't figure out what controls dormancy because we don't really know the defining events of dormancy. Most seed populations don't complete the process of dormancy release in a synchronous fashion, thus there is large variability in a population of seeds. Additionally, the events required for dormancy release may occur in relatively few cells in the embryonic axis, thus making it difficult to observe the real changes occurring. Since the seed is an entire package of embryo, endosperm and testa, dormancy should be studied on a whole-seed basis.

Is dormancy a lack of some key cellular event or an imposed event that must be negated before germination can be completed? Is the release from dormancy mediated through a common signal transduction chain that coordinates diverse cellular responses? It has been suggested that there are related or common receptors for dormancy-breaking agents within the plasma membrane of the responsive embryonic cells. When triggered, these receptors then initiate a signal transduction cascade, perhaps involving synthesis of or sensitization to germination-promoting substances, that leads to the completion of germination.

Probably the most widely studied aspect of dormancy regulation is regulation via plant hormones. In this section we will review the singular and interactive effects of the known plant hormones on the release of seeds from dormancy.

ABA

There is a tremendous amount of circumstantial evidence that ABA regulates the onset and maintenance of seed dormancy (Kucera *et al*., 2005). There is an incredible lack of understanding how it does so and no receptor for ABA has been identified (Finkelstein *et al*., 2002).

When seeds are dispersed from their parent, they are in a state of primary dormancy which is induced by ABA produced by the embryo during their development in the fruit. The level of ABA is low during the initial stages of seed development, it increases substantially during mid-development, and then declines during maturation and desiccation. Dormancy is only initiated when the embryo itself produces ABA as ABA produced by maternal tissue does not induce dormancy (Karssen *et al*., 1983; Groot and Karssen, 1992; Koornneef and Karssen, 1994; Hilhorst, 1995; Frey *et al*., 1999; Nambara and Marion-Poll, 2003).

Precocious seed germination and vivipary (germination before release from the parent) are usually associated with a deficiency of ABA or deficiency in sensitivity to ABA (Kucera *et al*., 2005). After-ripening is a period of dry storage at room temperature of freshly harvested mature seeds. It is a common process for releasing dormancy. ABA content and seed sensitivity to ABA declines and sensitivity to gibberellins increases as after-ripening proceeds (Kucera *et al*., 2005). ABA inhibits phase III water uptake, endosperm rupture, further embryo extension and seedling growth after radicle emergence (Kucera *et al*., 2005).

GA

While ABA induces dormancy during seed maturation, gibberellins play a key role in release from dormancy and promotion of germination. Even though there is considerable gibberellin biosynthesis in developing seeds, gibberellins seem to be involved with embryo and fruit growth rather than dormancy.

A high gibberellin:ABA ratio favors vivipary, adding support to the idea that gibberellins promote germination. Two key functions for gibberellins during germination have been suggested: (i) promoting embryo growth; and (ii) weakening tissues surrounding the radicle, thereby enhancing emergence.

Some seeds such as lettuce (*L. sativa*) are photodormant and require exposure to red light for germination. Exposure to red light transforms phytochrome from the red form to the physiologically active far-red form, inducing gibberellin production, thereby promoting germination.

Dormancy release followed by germination is the result of a balance between many promoting and inhibiting factors which target both the testa and the embryo. The main promoter is gibberellin while the main inhibitor is ABA. It seems that the gibberellin requirement for dormancy release and germination depends on the amount of ABA produced in the developing seeds which sets the level of dormancy as well as the amount of ABA produced upon imbibition which maintains the dormancy already set. It appears that gibberellin levels are always adequate for germination but can only lead to it if ABA synthesis is inhibited. The transition through dormancy to germination is accompanied by decreased sensitivity to ABA and increased sensitivity to gibberellins as well as a reduction in synthesis of ABA.

Ethylene

Ethylene production increases with germination of many seeds and ethylene production is often higher in non-dormant compared with dormant seeds. Even though ethylene seems to promote dormancy release and germination, no clear role in either process has been established (Kucera *et al*., 2005).

Brassinosteroids

Brassinosteroids in general, promote germination by acting in conjunction with gibberellins to promote cell elongation and counteract the inhibitory effects of ABA. Brassinosteroids may also stimulate gibberellin biosynthesis.

Cytokinins

Cytokinins are present in developing seeds, predominantly in the endosperm, and are known to break seed dormancy in many species by enhancing ethylene production. Cell division, especially during root elongation, is also promoted by cytokinins.

Auxins

Auxins play a major role in embryogenesis as previously discussed. During germination, auxin regulates catalase activity which is important in getting rid of toxic metabolites.

Summary

The effects of hormones on seed dormancy and subsequent germination has been studied extensively. Even so, all that work can be summarized into a very brief space regarding what we really know. ABA induces and maintains dormancy, and for a prolonged effect, it must come from the embryo. Gibberellins promote release from dormancy and germination by counteracting the inhibitory effects of ABA. The roles that brassinosteroids, ethylene, auxins and cytokinins play are still being explored.

6 Some Abiotic Plant Stressors – Oxygen, Minerals, and Salt

Plants are subjected to enormous biotic and abiotic stresses during their lifetime (Fig. 6.1). No matter what the source of the stress, the impact is ultimately at the cellular level, eventually manifesting itself at the whole plant level. This manifestation is often seen as reduced performance in the field, reflected in reduced yield or quality.

The basis for many plant responses to stress revolves around a cells' relationship with oxygen. This is a major paradox of biology. While oxygen is required to sustain life, in a slightly altered form, oxygen can wreak havoc on life processes. This chapter explores the many faces of oxygen, the physiology of oxidative stress in plants, and the protective role of antioxidants in plants. In addition, stresses imposed upon plants by minerals and salt are explored.

Forms of Oxygen

Most of us think of oxygen as an essential component of respiration and a wonderful product of photosynthesis. Oxygen also has a dark side. In order to understand the dark aspect of oxygen, we need to understand its dangerous, mutant forms, reactive oxygen species (ROS) (Table 6.1).

Atmospheric oxygen (O_2), also called triplet oxygen, in general does not oxidize (remove electrons from) organic substances. It is the stable form of oxygen we are familiar with. It has two unpaired electrons with parallel spins, and this parallel spin prevents reactivity. In general, in order to participate in chemical reactions with organic molecules oxygen must be activated. Activation can occur through the reversal of the spin of one of the unpaired electrons by absorption of energy to form singlet oxygen (O_2^1). Singlet oxygen is very reactive with many organic molecules and can be involved in divalent reduction (transfer of two electrons at once). Activation can also occur by adding electrons to O_2. If an oxygen molecule (O_2) gains one electron, it becomes a superoxide radical (O_2^-). This process is endothermic, absorbing heat as it occurs. If (O_2) gains two electrons, it forms hydrogen peroxide (H_2O_2), and if it gains three electrons, it forms a hydroxyl radical (HO^-). Both of these reactions are exothermic, releasing heat as they take place (Afanasev, 1985).

These altered forms of oxygen are capable of unrestricted oxidation (removal of electrons from) of cellular components which can ultimately result in cell death. Cell death may occur due to peroxidation of membrane lipids, oxidation of proteins, enzyme inhibition, and nucleic acid damage. ROS also act as signaling molecules in plants, leading to the activation of metabolic pathways for stress resistance.

H_2O_2 is a particularly important ROS. It passes through membranes easily and is not compartmentalized within the cell. H_2O_2 is also involved in the formation of many complex organic molecules. Even though H_2O_2 is known to be very reactive, it alone is not that impressive. However, in the presence of a metal reductant (electron donor), it forms the highly reactive hydroxyl radical which is the strongest oxidizing agent known.

Sources of These Altered Forms

Many ROS are byproducts of photosynthesis and respiration (Gill and Tuteja, 2010). In the light, most ROS come from chloroplasts and peroxisomes (Foyer and Noctor, 2003) while mitochondria produce most of the ROS formed in the dark (Moller, 2001). They are also produced during abiotic stress, pathogen defense or normal senescence. A normal rate of ROS production in cells is 240 μM O_2^-/s which includes around 0.5 μ μM H_2O_2/s. Under stressful conditions production can increase to 700 μM O_2^-/s with up to 5–15 μM H_2O_2. Increased ROS production can be caused by drought, chilling, heat, high light, desiccation,

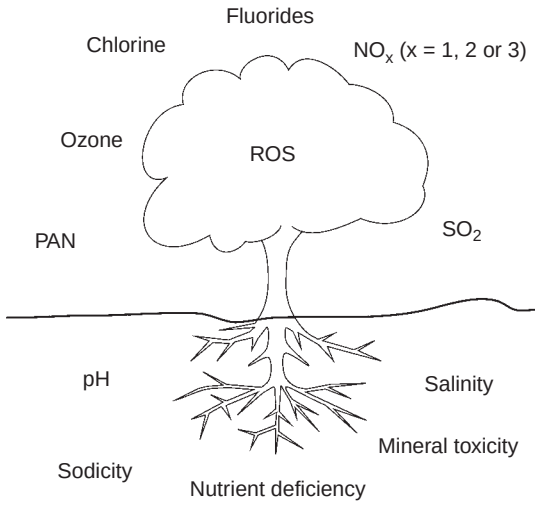

Fig. 6.1. The many abiotic plant stressors. PAN, Peroxyacetylnitrate; ROS, reactive oxygen species.

Table 6.1. Forms of oxygen important in plant physiology.

Form of oxygen	Molecular formula	How it is formed
Atmospheric oxygen (triplet oxygen)	O_2	
Singlet oxygen	O_2^1	Reverse the spin of one of the unpaired electrons in O_2 by adding energy
Superoxide radical	O_2^-	Add one electron to O_2
Hydrogen peroxide	H_2O_2	Add two electrons to O_2
Hydroxyl radical	HO^-	Add three electrons to O_2

heavy metals, salt, ultraviolet (UV) radiation, nutrient deficiencies, air pollution (ozone and sulfur dioxide, SO_2), pathogen attack, and mechanical stress. Since ROS can be both beneficial (in the case of signaling for pathogen defense) and harmful (as in protein or lipid oxidation), ROS levels must be precisely regulated at the cellular level.

Eliminating ROS

Excessive amounts of ROS must be eliminated from plant cells to avoid damage. This is accomplished either through direct elimination via specific metabolic pathways or by avoiding or preventing their production in the first place.

The major ROS scavengers in plants are enzymes that include superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) (Gill and Tuteja, 2010). Their balance and activity is important in regulating the levels of superoxide radicals and H_2O_2. This balance coupled with heavy metal ion sequestration is important for avoiding the formation of the highly toxic hydroxyl radical.

The most effective enzymatic antioxidant is SOD, which is found in all cells (Beyer *et al*., 1991). Its activity depends on metal cofactors including copper, zinc, manganese or iron. As the name implies, SOD is responsible for the dismutation of the superoxide molecule. Dismutation is a simultaneous reduction and oxidation of a substance to produce two different products. Superoxide molecules are dismuted into O_2 and H_2O_2.

APXs are a group of enzymes responsible for reducing H_2O_2 to H_2O by transferring an electron from H_2O_2 to ascorbate. In the process dehydroascorbate is produced, which is reduced to ascorbate at the expense of creating oxidized glutathione. The oxidized glutathione is reduced using an electron from nicotinamide adenine dinucleotide phosphate (NADPH). The overall process is the transfer of an electron from NADPH to H_2O_2 through glutathione and ascorbate, reducing the H_2O_2 to H_2O and making it harmless. This is called the glutathione-ascorbate cycle and is important for removing H_2O_2 from plants.

CATs are a group of enzymes with the ability to dismutate H_2O_2 into H_2O and O_2. CAT is a very effective antioxidant enzyme: one molecule of CAT can dismutate millions of molecules of H_2O_2/min.

In addition to enzyme-mediated removal of ROS, antioxidants such as ascorbic acid and glutathione are important in a plant's defense against oxidative stress.

Rather than scavenging ROS during stress conditions, avoiding their production in the first place is an effective mechanism for avoiding damage. Some mechanisms that help reduce ROS production during stress include anatomical, physiological and metabolic adaptations. Anatomically, leaf curling in corn (*Zea mays*) to reduce water loss during drought stress may also help reduce ROS production. Physiologically, C4 and CAM metabolism

alter 'normal' photosynthesis by incorporating additional steps into carbon fixation. This helps prevent the transfer of electrons to O_2 instead of CO_2 that often occurs in C3 metabolism. The rearranging of the photosynthetic machinery in response to light quality and quantity represents a molecular mechanism to balance light absorption with CO_2 availability. This prevents the production of excess electrons under high light levels thus reducing the transfer of electrons to O_2.

The production of ROS in chloroplasts and mitochondria can be reduced by enzymes called alternative oxidases (AOX). These enzymes channel electrons flowing through electron transport chains of photosynthesis and respiration to O_2, producing water instead of O_2^-. The levels of ROS are decreased in two ways: (i) by producing water instead of O_2^-; and (ii) by reducing the level of O_2 available for further ROS production.

ROS and Plant Defense

ROS are a key cellular signal to the plant that it is under attack by a pathogen. As a pathogen attacks a plant, ROS are produced in plant cells through enhanced activity of plasma-membrane-bound NADPH oxidases, amine oxidases located in the apoplast, and peroxidases bound to the cell wall. H_2O_2 is produced during this response and seems to diffuse into cells and along with the plant hormone salicylic acid, activates many of the plant defenses against the pathogen.

Salicylic acid at the same time suppresses the activity of ROS-scavenging enzymes, allowing ROS levels to increase indicating the attack. Without this reduction in ROS-scavenging activity, ROS levels could never reach a level needed to signal the need for defense.

The function of ROS is very different during a biotic attack compared with an abiotic attack. During the biotic attack, defenses are signaled via accumulated ROS while during an abiotic stress, increased ROS levels summon ROS scavengers to decrease high levels of ROS produced during the stress. But what happens if a plant comes under a biotic attack while undergoing an abiotic stress such as drought? Plants that are under an abiotic stress before or during a biotic attack generally develop less resistance to the biotic attack than plants not under an abiotic stress before or during the attack.

Plant Antioxidants

Ascorbic acid

Vitamin C (L-ascorbic acid, ascorbate) is plentiful in plant tissues (Foyer, 1993). It has received much attention as an important human nutrient. However, little attention has been given to its importance in plant health. Ascorbate, which is directly produced through conversion of D-glucose, is an important plant antioxidant, found mostly in the chloroplast, directly scavenging free radicals to minimize oxidative stress in plants (Smirnoff, 2005). Ascorbate is also capable of indirect ROS scavenging by reducing (adding an electron to) tocopherol, which in its reduced form is a ROS scavenger. Much of a plant's ascorbate is localized within the chloroplast, where it scavenges H_2O_2. In addition, ascorbate may also be important for cell wall biosynthesis.

Glutathione

Glutathione, a tripeptide, has antioxidant function due to the sulfydryl group of cysteine, one of the three amino acids which form the molecule. Most glutathione exists in the reduced form, in the chloroplast and cytosol, and its concentration declines with tissue age (Larson, 1988; Alscher, 1989).

Glutathione can effectively react with singlet oxygen, superoxide and hydroxyl radicals, functioning directly as a ROS scavenger. Glutathione might also help protect membrane integrity by getting rid of products of lipid peroxidation. It is also the reducing agent during recycling of ascorbate from its oxidized to its reduced form.

Glutathione also has other cellular functions besides acting as a ROS scavenger: (i) it appears to help in sulfur transfer from source to sink tissues; (ii) it helps detoxify xenobiotics (substances not normally found in an organism, or found in much higher than normal concentrations); (iii) it is a precursor to heavy metal binding chelates in plants; and (iv) it confers tolerance of corn (*Z. mays*) plants to the herbicide triazine.

Tocopherol

The tocopherols, and particularly the most active α-tocopherol (vitamin E), are a well-known family of antioxidants located exclusively in cell membranes (Diplock *et al.*, 1989; Fryer, 1992; Hess, 1993). They are particularly effective in quenching singlet oxygen and peroxides (Kamal-Eldin and

Appelqvist, 1996). Tocopherols are found predominantly in plants in chloroplast thylakoid membranes, thus leaves are a particularly rich source. α-Tocopherol also stabilizes membranes by sequestering free fatty acids which can act like detergents disrupting membrane integrity.

Carotenoids

Carotenoids are 40 carbon isoprenoids and tetraterpenes located in plastids of all plant cells. They are accessory pigments in chloroplasts and also detoxify activated oxygen and triplet chlorophyll that are produced during light harvesting in photosynthesis (Peñuelas and Munné-Bosch, 2005).

The two classes of carotenoids include carotenes and xanthophylls. Carotenes are hydrocarbon carotenoids and xanthophylls are derived from carotenes that contain one or more oxygen atoms. Carotenoids exist in the ground state or in one of two excited states during light harvesting.

Carotenoids function as antioxidants in many ways (Young, 1991). They effectively terminate lipid peroxidation chain reactions and thereby reduce damage to membranes by ROS. They scavenge singlet oxygen and dissipate the energy as heat. This is particularly important when light levels are above the saturation level and singlet oxygen is plentiful. Carotenoids, especially β-carotene, react with triplet or excited chlorophyll preventing the formation of singlet oxygen. Finally, cycling of xanthophyll between two forms, violaxanthin and zeaxanthin, dissipates excess energy formed by both photosystems I and II (Demmig-Adams and Adams, 1993).

Proline

Proline is an effective antioxidant (Chen and Dickman, 2005) as well as an important osmolyte. Though proline is most often associated with osmotic adjustment occurring during drought stress, it is also a potent ROS scavenger (Ashraf and Foolad, 2007; Trovato *et al*., 2008). Proline is especially important in quenching ROS produced by drought, salt or heavy metal stress.

Flavonoids

Flavonoids are a group of polyphenolic compounds found especially in leaves and flowers which have a variety of functions in plant metabolism, including functioning as powerful antioxidants. Their ring structure makes them particularly effective in neutralizing ROS. Flavonoids are also important in signaling resistance to pathogens and may also act as feeding deterrents (Gould and Lister, 2006).

Herbicides and ROS

Several of the herbicides widely used in agriculture function via the production of ROS. Some plants have developed resistance to these herbicides by increasing their ability to scavenge for ROS.

Paraquat and diquat, both bipyridylium (viologen) herbicides, are non-selective herbicides applied to leaves inducing rapid wilting and leaf desiccation followed by necrosis within 24 h (Calderbank, 1968). Both herbicides require light and chlorophyll to induce injury. The first cellular signs of injury include chloroplast swelling, quickly followed by tonoplast and plasmalemma breakdown.

Paraquat's toxicity relies on its ability to very effectively generate ROS. When an electron is donated to the paraquat^{2+} molecule (the original bipyridylium divalent cation), the free radical paraquat^{1+} is formed. An electron is transferred from paraquat^{1+} to oxygen, regenerating paraquat^{2+} and a superoxide radical. Further reactions convert the superoxide radical to a hydroxyl radical.

The electron donated to the paraquat^{2+} molecule comes from the primary electron acceptor in PSI, ferredoxin. Since other reducing agents are also effective electron contributors to paraquat^{2+}, all other living organisms subject to ROS damage are also injured by paraquat, and this includes humans. Resistance to paraquat has been generated by a number of weed species including perennial ryegrass (*Lolium perenne*). The resistance is the result of increased ROS-scavenging enzymes (Harper and Harvey, 1978; Fuerst and Vaughn, 1990).

Some herbicides, the p-nitrodiphenyl ethers and aminolevulinic acid-based modulators, work by causing the accumulation of intermediates involved in chlorophyll production called tetrapyrroles. Light energy is absorbed by the tetrapyrroles which is then used to create toxic singlet oxygen. Since chlorophyll production is inhibited, the leaves become bleached. Wilting, desiccation, and finally necrosis soon follow.

Cellular Activity of ROS

The reactions of ROS with cellular components are complex. Consideration must be given to: (i) the cellular component in question; (ii) its location within the cell; (iii) the ROS interacting with it; (iv) electrical charges; (v) membranes; (vi) macromolecular binding; and (vii) compartmentalization of enzymes, substrates, and catalysts. While the effects of ROS at the cellular level are complex, examining their general effects on lipids and proteins emphasizes their potential impact on plants.

Lipid peroxidation

Lipids are an integral part of cellular membranes and are particularly important for cellular compartmentalization and integrity (Frankel, 1985). Oxidation of lipids has been extensively studied since they are extremely important in the development of rancid and undesirable flavors in food products.

The oxidation of lipids, more appropriately called lipid peroxidation, is the process in which free radicals 'steal' electrons from fatty acids within the lipid molecules causing damage. Polyunsaturated fatty acids are most vulnerable because they have many double bonds with intervening methylene (–CH2–) groups with especially reactive hydrogens. The process is a chain reaction event with an initiation, propagation, and termination.

Lipid peroxidation is initiated when a ROS combines with a hydrogen atom from a fatty acid to produce water and a fatty acid radical. This fatty acid radical is not a very stable molecule, so it reacts quickly with molecular oxygen to create a peroxy-fatty acid radical, which is also unstable. This reacts with another fatty acid to produce a new fatty acid radical and a lipid peroxide or a cyclic peroxide if it reacted with itself. The cycle continues with a chain reaction effect since a radical is always produced when a radical reacts with a non-radical. The chain reaction stops only when two radicals react to produce a non-radical, which only occurs when the concentration of radicals is extremely high. Termination may also occur if the radicals are caught by antioxidants such as vitamins E or C or the enzymes SOD, CAT, and peroxidase.

The damage to the membrane is a reduction in integrity and function, with cell death often following. In addition, toxic end-products can be mutagenic and carcinogenic.

Protein oxidation

Oxidation of proteins results in changes in protein structure, which changes function. Fragmentation of the peptide chain, altered electrical properties, and increased susceptibility to proteolysis may also occur (Davies, 1987). Amino acids differ in their susceptibility to oxidation and the ROS differ in their effects on specific amino acids. Most changes in a protein due to oxidation are not reversible. DNA is particularly sensitive to oxidation since any change in the molecule can lead to enormous dysfunction. Deletions, mutations and other lethal changes in the DNA molecule occur because of oxidation. Both the sugar and the base portions of the molecule are susceptible to oxidation (Imlay and Linn, 1986).

Air Pollution

Air pollution exists in many forms, all with potential impacts on plant growth and productivity. Most air pollutants are products of human activity including, but not limited to: (i) manufacturing; (ii) use of the internal combustion engine; and (iii) burning of organic materials including wood, coal and oil (Zeiger, 2010). Some pollutants resulting from human activity include CO_2, CO, H_2S, HF, SO_2, NO, NO_2^-, C_2H_4, and particulates. Complex chemical reactions in the atmosphere between sunlight and certain of these substances lead to the production of ozone, peroxyacetylnitrate (PAN) and H_2O_2. Exposure to any of these substances can negatively impact plant growth and development (Zeiger, 2010; Gheorghe and Ion, 2011) and the plant responses to these pollutants may be acute or chronic.

While over 3000 substances have been identified as air pollutants (Gheorghe and Ion, 2011) a more limited number have been confirmed to affect plant growth. Pollutants can have a direct, toxic effect on plants or they may have an indirect effect by altering soil pH and heavy metal availability in the soil (Gheorghe and Ion, 2011).

To understand the impact of these pollutants on plant growth and development, long-term studies are needed. Short-term studies subjecting plants to high levels of specific pollutants for short periods of times can produce abnormal results that would not reflect long-term exposure to lower levels of pollutants. Metabolism is altered long before visible symptoms appear. For example, photosynthesis

is inhibited with as little as 0.1 ml NO_x/l while visible necrosis is not visible until levels reach 5 ml/l (Zeiger, 2010). When other pollutants are added to the mix, the threshold for metabolic disorder is even lower. Furthermore, weakened tissues are more susceptible to injury from other sources such as drought, heat or biotic pests.

Particulates

While often not being directly toxic to plants, particulate pollutants such as dust generally reduce photosynthesis by reducing light interception by leaves and clogging stomata, lowering their conductance to CO_2 (Zeiger, 2010).

There are many forms of particulates that are considered potentially harmful to plants (Gheorghe and Ion, 2011) and many of them elicit their effects only on plants close to the point source of the pollutant. Dust from cement factories contains large amounts of calcium oxide which is alkaline so when it is dissolved in atmospheric moisture it can have a pH as high as 12 (Gheorghe and Ion, 2011). Other pollutant dusts include lime, gypsum, soot, magnesium oxide, boric acid, potassium, calcium and sodium chlorides, and sodium sulfate.

Pubescent leaves tend to trap more dust particles than leaves that are glabrous. In either type of leaf, dust particles may be quite abrasive and may erode a leaf's cuticle. In addition, when dust that has been deposited on a leaf's surface becomes wet and then dries, a crust may form which is difficult to remove.

SO_2 and NO_x

Both SO_2 and NO_x (x = 1, 2 or 3, depending on specific situation) react with water to form acid rain. 'Pure' rain is slightly acidic with a pH of about 5.6, due to carbonic acid (H_2CO_3) resulting from dissolved CO_2. NO_x and SO_2 dissolved in rain decreases its pH to between 3 and 4. This acidity can remove minerals from plant tissues and affect soil pH which must be corrected with the addition of a buffering agent, usually limestone, in areas where the soil is low in calcium carbonate.

Both SO_2 and NO_x are absorbed via the same route as CO_2 and when dissolved in water inside the leaf, may form NO_2^-, which at high levels is toxic. Absorption of SO_2 can cause stomatal closure which reduces photosynthesis. In the cell, SO_2 dissolves to form bisulfite and sulfite ions. Sulfite can be toxic at high concentrations. It can also be metabolized to sulfate, providing a source of sulfur to the plant.

Leaves exposed to SO_2 may exhibit irregular white, red, brown or black spots (Gheorghe and Ion, 2011). In general, younger leaves are more sensitive than older leaves to damage and seedlings are more vulnerable than older plants. If SO_2 concentrations are high enough, the thylakoid membrane in chloroplasts begins to swell and electron transport is interrupted. This ultimately leads to reduced photosynthesis. In addition, respiration increases with exposure. SO_2 can also affect protein and membrane structure. Visible injury symptoms can be acute or chronic. Acute symptoms appear as lesions on both sides of the leaves which start out as interveinal or marginal water-soaked regions which quickly turn white, tan or reddish brown depending on the time of year and species. Chronic injury symptoms are a general chlorosis and occasional bronzing on the underside of the leaves.

Some species particularly sensitive to SO_2 include alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), buckwheat (*Fagopyrum esculentum*), clover (*Trifolium* spp.), oats (*Avena sativa*), pumpkin (*Cucurbita* spp.), radish (*Raphanus sativus*), rhubarb (*Rheum rhabarbarum*), spinach (*Spinacia oleracea*), squash (*Cucurbita* spp.), Swiss chard (*Beta vulgaris*), and tobacco (*Nicotiana tabacum*). Species more resistant to SO_2 injury include asparagus (*Asparagus officinalis*), cabbage (*Brassica oleracea* Capitata Group), celery (*Apium graveolens*), corn (*Z. mays*), onion (*Allium cepa*), and potato (*Solanum tuberosum*).

Nitrogen dioxide (NO_2) is a gaseous air pollutant which mainly affects younger plants and younger tissue, especially leaves. NO_2 induces the formation of crystalline structures in the stroma of the chloroplast which leads to swelling of the thylakoid membrane and a reduction in photosynthesis. Chlorosis and tip burn of leaves or needles are common symptoms.

Ozone

Smog produced through complex interactions of sunlight with some of the aforementioned chemicals contains the well-known pollutants ozone (O_3) and PAN. These pollutants can injure both plants and animals. One of the most noxious and phytotoxic pollutants is ozone (Heagle, 1989). It is a

highly reactive, irritating gas which can directly bind to membranes and interfere with metabolism. It also reacts with oxygen to produce ROS, which then impart their dysfunctions.

So how big of a problem is ozone pollution? Initially, the impact on crops and productivity was thought to be limited to areas near sources of the pollutant, such as power plants, urban centers, and industrial sites (Fiscus *et al*., 2005). However, as industrialization continues, populations increase and automobile contributions to the ozone problem steadily increase, the problem has taken on a global scale (Krupa *et al*., 1995). The average tropospheric ozone level worldwide is approximately 50 nmol/mol (the year 2000; Fiscus *et al*., 2005), 25% above the level established as harmful to sensitive species (Fuhrer *et al*., 1997). A huge problem is that many ozone precursors are emitted in automobile exhaust.

Ozone enters a leaf through stomata and then dissolves in the aqueous layer surrounding the cells producing hydroxyl and peroxyl radicals along with superoxide, albeit very slowly (Heath, 1987). Additionally, ozone may react directly with membrane lipids, amino acid in membrane proteins or enzymes encountered in the cell wall (Fiscus *et al*., 2005).

Products of these early ozone reactions are probably the messengers leading to the plant's response to ozone exposure. These initial messengers probably interact with other plant defense messengers such as ethylene, salicylic and jasmonic acid. Ethylene seems to promote ozone injury while jasmonate seems to help minimize injury from ozone (Fiscus *et al*., 2005).

After the initial reactions with ozone in the apoplast, further oxidation occurs in the cytoplasm and subcellular components ultimately resulting in visible injury seen as lesions. If exposure to ozone is high, lesion formation is acute, with lesions becoming visible within hours of exposure. At lower doses, lesion formation occurs more slowly over a period of days or even weeks.

Ozone interferes with normal photosynthesis by interfering with electron transport, guard cell function and by reducing ribulose bisphosphate carboxylase oxygenase (RuBisCO), levels and activity (Miller, 1987). Transport of photosynthates to sinks from the source leaf may also be impaired by ozone, further reducing photosynthesis via inhibitory feedback mechanisms. Ozone exposure generally has a more deleterious effect on yield when the exposure occurs during the reproductive period compared with the reduction seen with exposure during vegetative growth.

While a general overall reduction in productivity is observed with exposure to ozone, the specific amount of reduction varies considerably among species, cultivars within species, length and level of exposure, environmental conditions during exposure and concomitant exposure to other pollutants. Specific environmental factors affecting the response to ozone include: (i) leaf temperature; (ii) water vapor pressure deficit between the leaf and air; (iii) photosynthetic photon flux density (PPFD); (iv) soil water availability; and (v) atmospheric CO_2 concentration.

The same mechanisms for dealing with oxidative stress are involved in ozone tolerance. In particular, ascorbic acid synthesized in the cytoplasm and transported to the apoplast seems to be quite important in detoxifying ozone and related ROS.

PAN

PAN (peroxyacetylnitrate, $C_2H_3NO_5$) is a photochemically produced secondary pollutant. It is a major component of smog and may cause injury at levels as low as 1 ppm. Symptoms on sensitive species appear as silvering or bronzing of lower leaves (Thomson *et al*., 1965). As a toxin, PAN interferes with photosynthesis and cell wall metabolism.

Fluorides

Fluorides are toxic pollutants produced in the manufacturing of brickwork, aluminum, glass, steel, ceramics, and phosphate fertilizer plants. Fluorides may be in the gaseous form (HF, SiF_6, CF_4, F_2) or occur as particulates (Ca_3AlF_6, cryolite), CaF_2, NH_3F, AlF_6, CaSiF, NaF and Na_2SiF_6. Aerosols of NaF, $NaAlF_6$ and AlF^6 may also form (Gheorghe and Ion, 2011). The most injurious form of fluoride is HF. Particulate fluorides fall on plants and gaseous fluorides are absorbed by plants causing injury to both plants and animals. Cattle are particularly sensitive to ingested fluoride from particulate pollution of forage crops (Shupe, 1969).

Plants are sensitive to levels as low as 0.1 ppm HF, the main source of plant injury. If the source of fluorine is eliminated, many plants will recover from injury. Fluorides combine with the metallic component of enzymes and inhibit their functions. General cellular metabolism is impaired, and in

particular, photosynthesis, respiration, nucleic acid synthesis and energy production are affected. In leaves exposed to HF, ribosomes detach from the endoplasmic reticulum, the number of ribosomes decrease and mitochondria swell. Ultimately tissue necrosis occurs, particularly in leaf margins. Species particularly susceptible to fluoride injury include apricot (*Prunus armeniaca*), blueberry (*Vaccinium* spp.), peach (*Prunus persica*), gladiolus (*Gladiolus communis*), grape (*Vitis* spp.), plum (*Prunus* spp.) sweet corn (*Zea mays*) and tulip (*Tulipa* spp.). Resistant species include alfalfa (*M. sativa*), asparagus (*A. officinalis*), bean (*Phaseolus* spp.), cabbage (*B. oleracea* Capitatia Group), carrot (*Daucus carota*), cauliflower (*Brassica oleracea* Botrytis Group), celery (*A. graveolens*), cucumber (*Cucumis sativus*), eggplant (*Solanum melongena*), pea (*Pisum sativum*), pear (*Pyrus* spp.), pepper (*Capsicum* spp.), potato (*S. tuberosum*), squash (*Cucurbita* spp.), tobacco (*N. tabacum*) and wheat (*Triticum aestivum*).

Chlorine

Chlorine is a toxic gas that may cause plant injury. Injury is often limited to the area immediately surrounding the site of discharge. The two main sources of chlorine pollution are: (i) chlorine gas (Cl_2); and (ii) hydrogen chloride (HCl), which quickly forms aerosols of hydrochloric acid (Gheorghe and Ion, 2011).

Older plants are generally more sensitive to injury from Cl_2 than younger plants but tissue age within a plant does not matter. Symptoms of injury are quite variable but often include tip burn of leaves or needles. Many species can recover from chlorine injury if the source of chlorine is eliminated.

Other atmospheric pollutants

Some other pollutants which may cause problems in limited situations include ammonia, volatile organic compounds, ethylene, methane, chlorofluorocarbons, hydrogen sulfide, bromine gas, carbon monoxide, iodine, and mercury vapor.

Ethylene is also a plant hormone, thus exposure to ethylene can cause a wide range of changes in plant metabolism. In general, plant growth is severely stunted with exposure to excessive amounts of ethylene. Leaf and flower abscission can also occur. Epinasty is also a common symptom of ethylene exposure.

Soil-related Stress

Soil is a complex mixture of biotic and abiotic components. The productivity or lack thereof of a given soil is often related to the nutrients available to plants for growth. Both the quantity and the quality or form of nutrients is important to consider. While a number of chemical attributes of a soil are important for helping determine their productivity, one of the most important attributes of a soil directly affecting naturally occurring or producer-applied nutrient availability is pH.

Soil pH

Soil pH is a key factor in determining the form, solubility, and availability of a nutrient in a soil and any problems that may be associated with its deficiency or excess. Soil pH is a measure of the acidity or alkalinity of a soil solution (the solution which results when a soil is wet) and may range from 0 to 14, 0 being acidic and 14 being basic. Low pH is associated with a high concentration of H^+ ions and low concentration of OH^- ions in the soil solution. A high pH is just the opposite. The concentration of H^+ ions helps determine the availability to the plant of nutrients in the soil if present. It is also associated with the overabundance of some nutrients which may be toxic (e.g. aluminum). Most soil-derived nutrients are readily available for plant use at a soil pH of between 5.5 and 6.5.

Soil pH changes over time. Soils will tend to become more acidic as rainwater, which itself is acidic, leaches basic ions such as calcium, magnesium, potassium and sodium out of the soil. Carbon dioxide from decomposing organic matter and root respiration can dissolve in soil water to form a weak acid. Stronger acids such as nitric and sulfuric can form from decaying organic matter or from oxidation of ammonium and sulfur fertilizers. To reverse the pH-lowering influence of these acids, lime is usually added to acidic soils on a regular basis. In addition to raising the soil pH, lime also provides calcium and magnesium. In certain situations, such as extremely arid environments, soil pH can be lowered by applying agricultural sulfur.

Essential plant nutrients

All plants require certain elements to complete their life cycle. An essential element is required for most plants to complete their life cycle and no

other element can substitute for it. The element must also be either directly incorporated into a plant metabolite or be essential for enzyme function. Some plants utilize other elements, namely sodium, cobalt, vanadium, and silicon. They are not included on the list of essential elements because other elements can substitute for them if they are deficient or they are only used by some but not all plants.

There are 16 elements that have been identified as essential for plant growth: carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, zinc, copper, boron, molybdenum, and chlorine (Table 6.2). Carbon, hydrogen and oxygen are derived from CO_2 and H_2O and are not considered mineral elements. All of the other elements are supplied in the soil solution. Six of them (N, P, K, Ca, Mg, and S) are required in relatively large quantities and are therefore called macronutrients. The other seven (Fe, Zn, Mn, Cu, B, Mo, and Cl) are required in much lower amounts and are therefore called micro or minor nutrients (Hodges, 2003).

It is often helpful to remember the essential nutrients for their general, main function in plant physiology: (i) structure; (ii) energy metabolism; (iii) charge balance; (iv) enzyme activation; and (v) electron transport. The three structural elements are carbon, hydrogen, and oxygen. The nutrients involved in energy metabolism include nitrogen, sulfur, and phosphorus. Charge balance in a plant is maintained by potassium, calcium, and magnesium. Finally, enzyme activators and electron transporters include iron, manganese, zinc, copper, boron, molybdenum, and chlorine.

Nutrients are either mobile or immobile in both the soil and the plant. In the soil, NO_3^-, SO_4^{2-}, BO_3^{2-}, Cl^-, and Mn^{2+} are extremely soluble in water and are very mobile. Other soluble nutrients may be adsorbed to soil particles and are therefore considered less mobile. These nutrients include NH_4^+, K^+, Ca^{2+}, Mg^{2+}, and Cu^{2+}. The other nutrients are either only slightly soluble or adhere tightly to soil particles. They include $H_2PO_4^-$, HPO_4^{2-}, and Zn^{2+}.

Nutrient mobility in plants is important as it provides a hint as to where you should look for deficiency symptoms first, older or younger leaves. Nitrogen, phosphorus, and potassium are highly mobile and zinc is fairly mobile, thus symptoms will appear on older leaves first. Sulfur, iron, manganese, copper, molybdenum, and chlorine are less mobile and symptoms generally appear on older leaves first. Calcium and boron are immobile and symptoms always appear on younger tissues first.

It is important to understand each nutrient's general function in plant physiology to understand why we pay so much attention to fertility when trying to optimize growing conditions for our crops. The following discussion provides a brief yet complete outline of the function of the major and minor plant nutrients as well as a general description of the symptoms of their deficiencies. Excesses are much less common, but certain common situations will be discussed. Toxic nutrients, primarily metals, will be covered later in this chapter.

When one or more essential nutrients are lacking, a mineral deficiency develops. While symptoms of the deficiency of a single element may be relatively easy to identify in many species, multiple deficiencies developing at once can be very difficult to identify. Additionally, symptoms of stress from other sources, including excesses of some nutrient(s), may confound the issue making identification of the problem even more difficult. A deficiency in one or more nutrients nearly always results in reduced yield, product quality, and sometimes plant death.

Identification of nutrient deficiencies (and excesses) is important for good horticultural management (Table 6.3). Remember that deficiencies of different nutrients often have the same symptoms, and plants are often under a continually changing environment which may mask or exacerbate problems.

Carbon (C)

Carbon is one of the most plentiful elements in living organisms. The source of carbon for plant growth and development is CO_2. Carbon has four valence electrons allowing it to bond to as many as four other atoms. Carbon can donate or receive electrons in bonding, giving it both acidic and basic qualities. The valence electrons are oriented in such a way as to allow complex three-dimensional carbon-to-carbon bonding and complex molecular formations. Linear and ring molecules are possible. The bonds between two carbon atoms or a carbon and a hydrogen atom are high energy bonds. Thus complex molecules with carbon in them (e.g. carbohydrates) can store a tremendous amount of energy in them. This energy can be released in respiration.

Table 6.2. Essential plant nutrients, characteristics, and functions in plants.

Nutrient	Main absorbed form	Mobility	Major properties/functions
Carbon	CO_2	–	Can donate or receive electrons in bonding; complex three-dimensional carbon to carbon bonding with complex molecular formations; linear and ring molecules possible; bonds between two carbon atoms or a carbon and a hydrogen atom are high energy bonds; complex molecules with carbon in them can store a tremendous amount of energy
Hydrogen	H_2O	–	Energy transduction; ion movement; pH regulation
Oxygen	CO_2	–	Powerful oxidizing agent; combines with many other elements; creates polar molecules, hydrogen bonds and acidic groups; important in chelate formation
Nitrogen	NO_3^-; NH_4^+	Mobile	In ring molecules, acts as the center for redox reactions and the amine nitrogen is important in metal binding; can act as an electron donor; peptide bonds in proteins permits helix formation in nucleic acids; adds asymmetry to molecules allowing for important chemical properties such as basicity, charge, chemical reactivity and structure; major component of proteins (enzymes); part of the chlorophyll molecule
Phosphorus	$H_2PO_4^{2-}$	Mobile	Integral part of proteins and lipids; important in energy metabolism (ATP); important for stress tolerance
Potassium	K^+	Mobile	Catalyst and an enzyme activator for many reactions in protein and carbohydrate metabolism; important in stomatal function and water relations; improves disease resistance and quality of fruits and vegetables
Calcium	Ca^{2+}	Immobile	Important component of cell walls, linking with pectins to enhance rigidity; important in membrane integrity; important signaling molecule
Magnesium	Mg^{2+}	Mobile	Central atom in the chlorophyll molecule; enzyme activator especially those involved in ATP metabolism, nucleic acid synthesis and carbon fixation; important in membrane structure, especially organelle membranes
Sulfur	Mostly SO_4^{2-} from the soil; some SO_2 through the leaves	Slightly mobile	Major constituent of cysteine, cystine and methionine (amino acids); important in electron transfer reactions; sulfhydryl groups (SH) often the reactive sites of enzymes or coenzymes; important in protein conformation; important component of odor and flavor compounds in *Allium* and *Brassica*
Iron	Fe^{2+}; Fe^{3+}	Immobile	Important catalyst in the formation of chlorophyll; important in oxidation–reduction reactions; location for electron transfer in many enzymes; important for electron transport in photosystem I of photosynthesis
Manganese	Mn^{2+}; Mn^{3+}	Immobile	Enzyme activator; important in chlorophyll synthesis; a major function of manganese is the removal of electrons from water during photosynthesis; important in respiration and nitrogen metabolism; important in lignin production
Zinc	Zn^{3+}	Immobile	Primarily an enzyme activator, acting as a cofactor for more than 200 enzymes
Copper	Cu^{2+}	Immobile	Important for its oxidation–reduction properties; catalyst in chlorophyll formation; important part of several enzymes
Boron	H_3BO_3	Immobile	Important in pollen tube germination and sugar translocation across membranes
Molybdenum	MoO_4^{2-}	Mobile	Important as an electron carrier in nitrogen reduction from NO_3^- to NH_4^+; required by *Rhizobium* for nitrogen fixation in legumes
Chlorine	Cl^-	Mobile	Important in photosynthesis; may be important for maintaining an electrical equilibrium in the plant

Table 6.3. Quick general nutrient deficiency identification based on general symptoms for key nutrients that may often be deficient. Those nutrients that in general are not usually deficient are not included.

Worst symptoms on older or younger leaves?	General symptoms	Likely deficient nutrient
Older leaves	All leaves light green; older leaves uniformly chlorotic, some may be brown or dead; stems with greatly reduced terminal growth	Nitrogen
Older leaves	Most leaves dark green; stunted growth overall; abnormal red and purple leaves and stems; spindly growth	Phosphorus
Older leaves	Older leaves have interveinal chlorosis beginning at tips; margins may cup upwards; some leaves may be whitish	Magnesium
Older leaves	Leaves mottled; necrotic leaf tips and margins; crinkled leaves; abnormally short internodes; weak stems with brown streaking	Potassium
Newer leaves	Dead or burnt looking, deformed terminal buds	Calcium
Newer leaves	Light green leaves that are never whitish or chlorotic; veins lighter than interveinal tissue	Sulfur
Newer leaves	Leaves chlorotic with green veins	Iron

When carbon is deficient or limited in availability (such as in a closed greenhouse in the winter), growth is greatly reduced. Under normal growing conditions, carbon supply to the plant is limited only by stomatal closure.

Hydrogen (H)

Hydrogen is powerful reducing agent (accepting electrons). It is the most plentiful element in the universe as far as we know. Free hydrogen is rather rare, but it is just about everywhere combined with other elements. It is supplied to the plant as water (H_2O) and is split during photosynthesis to provide oxygen and the powerful reducing agent, the hydrogen ion (H^+). The hydrogen cation is extremely important in plants for energy transduction, ion movement, and pH regulation.

Hydrogen is never deficient, thus there are no symptoms for hydrogen deficiency.

Oxygen (O)

Oxygen is a powerful oxidizing (giving up electrons) agent. The most common form of oxygen is a gas, O_2. Other forms exist as ROS (as previously discussed in this chapter). Oxygen also combines with many other elements in both living and nonliving things.

When oxygen forms covalent bonds with other atoms, it tends to draw electrons towards itself, creating polar molecules (molecules that have charged ends), hydrogen bonds, and acidic groups. Oxygen is also important in chelate formation as it shares electrons with metals, keeping them held to the chelating molecule.

Nearly all of the oxygen in plants is derived from CO_2 during photosynthesis.

If oxygen is deficient (as in waterlogged soils) aerobic respiration is curtailed, often resulting in anaerobic respiration and the production of toxic metabolites.

Nitrogen (N)

After carbon, hydrogen and oxygen, nitrogen is the nutrient used in the largest quantities of any of the remaining essential nutrients. It is also usually the most deficient nutrient, especially in nonleguminous species. It is taken up by plants as nitrate (NO_3^-) or ammonium (NH_4^+).

It exists in nature primarily as N_2. Nitrogen has five electrons in its outer shell: three are valence electrons with two that are unshared, which makes positively charged ions possible. In ring molecules, nitrogen acts as the center for redox reactions and the amine nitrogen is important in metal binding, such as the iron in cytochrome or the magnesium in chlorophyll. In many reactions, nitrogen can act as an electron donor.

Peptide bonds in proteins are limited in how they can rotate which permits helix formation in nucleic acids. In addition, nitrogen adds asymmetry to molecules allowing for important chemical properties such as basicity, charge, chemical reactivity, and structure.

Most of the nitrogen found in plants is in the fully reduced state, while most of the nitrogen taken up from the soil by plants is in the fully oxidized state (NO_3^-). Nitrate must be reduced after being taken up by the plant before it can be used in metabolism.

Nitrogen is a major component of proteins. Enzymes are proteins and enzymes run the show in biology. Nitrogen is also part of the chlorophyll molecule.

Nitrogen is a mobile nutrient, meaning that it can move in a plant after its initial fixation into a metabolic component. Since it is mobile, deficiency symptoms appear on older tissues first. Nitrogen deficient plants normally have uniformly yellowing older leaves and sometimes the veins and petioles take on a reddish hue. Under an extreme deficiency, leaves may turn nearly white. Even though younger leaves remain green due to nitrogen translocated from the older leaves, they become progressively smaller in size. Branching is greatly reduced and plants become rather spindly. Older leaves become more sensitive to drought stress and senesce quickly.

The "nice" thing about nitrogen deficiency is that it is easily and quickly reversed by adding nitrogen. Recovery occurs within days, and given enough time, most plants can completely recover.

Excess nitrogen can cause excessive vegetative growth at the expense of sexual reproduction. Thus if fruit is the desired commodity, yields are greatly reduced and maturity is delayed. Excessive vegetative growth can also lead to lodging. In addition, succulent growth resulting from excess nitrogen may also be more susceptible to pest attack.

Phosphorus (P)

Phosphorus is taken up by the plant as the phosphate anion, $H_2PO_4^{2-}$. It is an integral part of proteins and lipids and is important in energy metabolism (ATP). Phosphorus is important for good root development and stress tolerance. Phosphorus is mobile, therefore the deficiency symptoms are observed first in older leaves.

Phosphorus deficient plants are often severely stunted but dark green, often mistaken for unstressed, younger plants. Some species develop a pronounced purplish color on the stems, petioles and underside of the leaves. With severe deficiency, the leaves often look bluish gray. Older leaves may also develop brown, netted veining. Seed formation may also be inhibited.

Potassium (K)

Next to nitrogen, potassium is the nutrient in the greatest quantity by plants. Plants take up potassium as K^+ and some plants may take up more than they really need and this is called 'luxury consumption'. Potassium is dissolved in the cytoplasm and apoplast and is not part of any structural component in the plant.

Potassium is necessary in plants as a catalyst and an enzyme activator for many reactions in protein and carbohydrate metabolism. Potassium is also very important in stomatal function and water relations of plants. Potassium often improves disease resistance and can also improve the quality of fruits and vegetables.

Potassium is a mobile nutrient, thus deficiency symptoms appear on older leaves. Symptoms include a general interveinal chlorosis which progresses from the tip and margins towards the central vein of recently matured leaves. Necrosis may also occur. Veins normally remain green and leaves may appear crinkled and curled. There is normally a sharp delineation among green, yellow, and necrotic tissues. In some plants, the first symptoms are white speckles on leaf blades. Other symptoms of potassium deficiency include lodging in grains and accumulation of excess, non-protein nitrogen in forage crops.

The chlorosis caused by potassium deficiency cannot be reversed as with nitrogen deficiency.

Calcium (Ca)

Calcium is often the most abundant cation in plants and is taken up by the plant as Ca^{2+}. Calcium is an important component of cell walls, linking with pectins to enhance rigidity. Calcium is also important in membrane integrity and calcium levels in the cytoplasm often change as hormonal signals induce developmental changes in cells. Thus calcium is an important signaling molecule or secondary messenger in plant development.

Since lime is often regularly applied to soils that might exhibit a calcium deficiency (i.e. soils with a low pH), field deficiencies are usually rare. Symptoms of calcium deficiency often do not become apparent since low soil pH which normally induces calcium deficiency usually induces other problems which would limit plant growth before calcium could reach deficient levels. Greenhouse and hydroponic culture is a different story. Calcium

deficiencies often occur in these situations, so care must be taken to monitor plants closely.

Calcium is immobile therefore deficiency symptoms appear in young tissue. A classic symptom of calcium deficiency is the failure of terminal buds to develop properly as meristems die. Margins of leaves also become necrotic as a result of calcium deficiency. Other classic calcium deficiencies include: (i) cat-facing and blossom end rot in tomatoes (*Solanum lycopersicum*); (ii) bitter pit and cork spot in apples (*Malus domestica*); (iii) black heart of celery (*A. graveolens*); (iv) leaf tip burn in cabbage (*B. oleracea* Capitata Group) and lettuce (*Lactuca sativa*); and (v) cavity spot of carrots (*D. carota*).

Many of these calcium deficiency problems are due to poor translocation of calcium to developing tissues rather than a low supply of calcium from the soil. In blossom end rot of tomatoes (*S. lycopersicum*), for example, the incidence of the malady is governed primarily by the supply of water, and therefore calcium, to the young developing fruit. If rapidly growing young fruit are suddenly subjected to a water deficit, the distal end of the fruit will become soft and rotten looking. This appearance is due to a lack of calcium pectate solidifying cell walls. An excess of water can also reduce the translocation of calcium and induce symptoms.

Magnesium (Mg)

Magnesium is taken up by plants as Mg^{2+}. One major function of magnesium plants is that it is the central atom in the chlorophyll molecule, thus crucial in photosynthesis. Magnesium is also important as an enzyme activator, particularly in enzymes involved in ATP metabolism, nucleic acid synthesis, and carbon fixation. Magnesium is also important in membrane structure, especially organelle membranes.

Magnesium is mobile therefore deficiency symptoms appear on older leaves first. It is expressed as interveinal chlorosis with some puckering of the leaf blade. In grasses, whitish or yellow striping of leaves may occur. Magnesium and nitrogen deficiencies may appear similar; however, veins ultimately become chlorotic in nitrogen deficiency but not with magnesium deficiency.

Sulfur (S)

Sulfur may be absorbed as sulfur dioxide (SO_2) through the leaves, but most sulfur in plants is absorbed as sulfate (SO_4^{2-}) from the soil. Sulfur is a major constituent of three (cysteine, cystine, and methionine) of the 21 amino acids that form proteins in plants. Sulfur is important in electron transfer reactions and sulfhydryl groups (SH) are often the reactive sites of enzymes or coenzymes, as they are important in protein conformation. Sulfur is also an important component of odor and flavor compounds in various *Allium* and *Brassica* species.

Sulfur is slightly mobile with deficiency symptoms sometimes on older leaves but normally appearing on younger leaves. Plants appear uniformly chlorotic, weak and spindly. Sulfur deficient leaves are often narrow with their veins exhibiting more chlorosis than the lamina. Veins and petioles often show a distinct red color. Sulfur and nitrogen deficiency appear similar, however, sulfur deficiency is widespread over the entire plant while nitrogen deficiency is usually confined to older leaves. In addition, the red color often seen on the underside of leaves and on petioles is pinkish and less vivid than that which might be seen in nitrogen deficient leaves. Sulfur deficient leaves ultimately form necrotic spots near the petiole, become more erect, twisted and brittle.

Iron (Fe)

Iron is a divalent or trivalent cation absorbed via the roots or foliarly. It is translocated in plants as a chelate since it precipitates readily. It is an important catalyst in the formation of chlorophyll. It is also important in oxidation–reduction reactions, the relatively easy transformation from Fe^{2+} to Fe^{3+} and vice versa. With this property, iron is the location for electron transfer in many enzymes. Iron is important for electron transport in photosystem I of photosynthesis.

Most iron deficiency occurs on high pH soils since iron is readily available to plants at a low pH. It is immobile, thus deficiency symptoms are first observed on younger tissue. Extensive interveinal chlorosis occurs, with veins remaining green. Under severe deficiency, leaves often turn white.

In excessive concentrations, iron is toxic to plants. This is because iron reacts with oxygen in what are called the Fenton reactions, producing toxic oxygen radicals.

Zinc (Zn)

Zinc is taken up from the soil by plants as a trivalent cation, Zn^{3+}. It can be taken up foliarly as well.

Zinc is primarily an enzyme activator, acting as a cofactor for more than 200 enzymes.

Zinc is immobile and young tissues show the first symptoms of deficiency. Interveinal chlorosis is quickly followed by severe reduction in internode length, giving the plant a rosette appearance. Leaf margins often become puckered. Pitting may develop on the interveinal tissues of the upper surfaces of older leaves. Guttation is also apparent with zinc deficiency.

Zinc toxicity can also occur, especially at low soil pH. Much of the absorbed zinc in a plant is complexed with citric and malic acid in the xylem, thus unavailable to interfere with plant metabolism.

Zinc toxicity symptoms first appear in young tissue as a general chlorosis which may progress to reddening due to anthocyanin production. Toxic levels of zinc induce small, vertically oriented leaves. Growth of the main root is inhibited and lateral roots are fewer and shorter and may exhibit a yellow coloration. Elongation of cells in the stem is inhibited with high zinc levels.

Excess zinc can inhibit both photosystems I and II with a reduction in photosynthesis due to displacement of magnesium by zinc at the water-splitting site in photosystem II. In addition, RuBisCO activity is inhibited. The primary toxic action of zinc is the inhibition of ATP synthesis and therefore energy metabolism in plants. In addition, free radical generation increases along with the activity of enzymes which remove them from the plant.

Boron (B)

Boron is absorbed as H_3BO_3 and can be absorbed through the leaves. With many crops there is a fine line between deficiency, sufficiency, and toxicity with boron. Boron is important in pollen tube germination and sugar translocation across membranes.

Boron is immobile and young tissues show deficiency symptoms before older tissues. Terminal bud growth stops, and shoot growth may appear as a rosette at the terminal ends of shoots.

While boron is often a deficient nutrient in many soils, there are instances when the level of boron is elevated enough to be toxic, particularly in arid or semi-arid regions.

Much of the boron in soils is not readily available for uptake by plants. When in the soil solution, boron is predominantly boric acid ($B(OH)_3$), which is extremely mobile and is easily leached from the soil, taken up by plants or temporarily adsorbed to soil or organic matter.

Soils high in boron are often derived from: (i) marine evaporites or sedimentation; (ii) high levels of boron in irrigation water; or (iii) from wastes from mining, coal-fueled electricity generation, and chemical discharge from manufacturing. Sodium perborate is also a widely used household and industrial bleaching agent which can contaminate water discharged from waste-water treatment facilities.

Some of the most common symptoms of boron toxicity in plants are chlorotic and necrotic patches at the tips or along the margins of older leaves. However, there are species (*Prunus, Malus* and *Pyrus*) where boron is mobile in the phloem and is deposited in sinks (fruit), thus leaf symptoms do not appear. Roots do not normally exhibit symptoms of boron toxicity.

Species that are particularly sensitive to high soil boron levels include avocado (*Persea americana*), apple (*M. domestica*), and green bean (*Phaseolus vulgaris*). Moderately sensitive species include oat (*Avena sativa*), corn (*Zea mays*), and potato (*S. tuberosum*) while tolerant species include carrot (*D. carota*), alfalfa (*M. sativum*), and sugarbeet (*Beta vulgaris*). Other boron-tolerant species include: saltbush (*Atriplex* spp.), milkvetch (*Astragalus*), barley (*Hordeum*), wheat (*Triticum*), Indian mustard (*Brassica juncea*), and tall fescue (*Festuca arundinacea*). The general mechanism for high soil boron tolerance is reduced boron uptake and it is controlled by several additive genes.

Manganese (Mn)

Manganese is absorbed by plants as di- or trivalent cations. It can also be absorbed through the leaves. Manganese is an important enzyme activator and is important in chlorophyll synthesis. A major function of manganese is the removal of electrons from water during photosynthesis. It is also important in respiration and nitrogen metabolism. Manganese is also important for plant defense against fungal infection as it is important in lignin production.

Manganese is immobile and young leaves develop yellow streaks. Deficiencies are rare since soils are generally adequate in manganese and the amount plants require is very low.

Toxicity may occur, especially when soil pH is low or soil levels of manganese have been elevated

by human activity. Manganese is transported through the xylem as a free ion.

Toxicity symptoms first appear as necrosis on leaves, petioles and stems on older tissue. There may be general leaf bronzing accompanied by a shortening of internode length. Younger leaves may appear crinkled and terminal buds may die. Roots may turn brown or crack.

Excess manganese may reduce chlorophyll levels in leaves which could lead to decreased photosynthesis. Loss of stomatal function might also occur. Fewer cells form per leaf, resulting in smaller leaves.

Molybdenum (Mo)

Molybdenum is absorbed as MoO_4^{2-}. It is important as an electron carrier in nitrogen reduction from NO_3^- to NH_4^+. Molybdenum is required by *Rhizobium* for nitrogen fixation in legumes.

Molybdenum deficiency appears as nitrogen deficiency, for that in fact is what molybdenum's deficiency causes. In broccoli (*Brassica oleracea* Italica Group) and cauliflower (*B. oleracea* Botrytis Group) molybdenum deficiency results in a classic whiptail leaves, resulting from lack of lamina and vascular differentiation. Molybdenum can be toxic and is easy to spot since leaves turn bright orange.

Copper (Cu)

Copper is taken up by plants as Cu^{2+} and can be absorbed through leaves. It is important for its oxidation–reduction properties. It is a catalyst in chlorophyll formation and is an important part of several enzymes.

Deficiency symptoms are rare, but when they do occur they originate in young tissue since copper is immobile. Leaves are often yellow and stunted and soon develop a bluish-green tint. Petioles curl and leaves turn downwards.

Copper may also be toxic. Organic matter in the soil tends to keep excess copper sequestered from plants. Copper is transported through the plant mostly through the xylem. There, copper is almost 100% sequestered to amino acids making it unavailable for involvement in plant maladies. However, if levels are high enough or soil organic matter is low, excesses can occur.

A common symptom of excess copper is interveinal chlorosis which may take the form of white or cream-colored spots or lesions. Leaf margins and tips can become necrotic and foliage may turn reddish purple. Radicles of emerging seedlings may be short and have blunt tips with necrosis with a predisposition to fungal attack. Root hair production is also inhibited.

Copper can substitute for magnesium in the chlorophyll molecule, thus in excess copper conditions, chlorophyll content is reduced which results in a reduced photosynthetic capacity. Photosystem II is inhibited, increased breakdown of carotenoids occurs and free radical production increases. With increased free radicle activity, leaf senescence is accelerated. Excessive copper also inhibits ATPase activity of the plasma membrane, reduces plasma membrane integrity, and increases potassium leakage from the cell. Both cell division and elongation are reduced.

Chlorine (Cl)

Chlorine is taken up by plants as the chloride ion, Cl^-. It is important in photosynthesis and may be important for maintaining an electrical equilibrium in the plant.

Deficiency symptoms are very rare, but may include abnormally shaped leaves with interveinal chlorosis and wilting of young leaves. Older leaves may cup downwards and in severe cases, bronzing of the upper side of mature leaves may appear. Excess can lead to poor storage of potatoes (*S. tuberosum*) and reduced smoking quality of tobacco (*N. tabacum*). Some species (*Persea, Prunus, Vitis*) are particularly sensitive to even low to moderate chloride levels in the soil.

Mineral toxicity

Certain elements, especially metals, including aluminum, manganese, lead, and cadmium are particularly toxic to plants and are often most available (soluble) when the pH is less than 6. These toxic elements are often present in soils that are productive but they do not cause harm to plants because at productive soil pHs, the normally toxic elements are not soluble in the soil solution. In other cases, these metals are present at particularly toxic levels due to contamination of the soil or groundwater by human activity.

The general plant response to metal toxicity is reduced productivity. Ultimately this reduction is traced back to abnormal metabolism induced by one or more toxic metals. For example, photosynthesis is reduced with copper toxicity because

copper atoms replace magnesium in chlorophyll molecules. These chlorophyll molecules are photosynthetically ineffective and ROS begin to accumulate causing premature senescence of leaves. Zinc is also known to inhibit photosynthesis by replacing magnesium at the site of water splitting in photosystem II. Heavy metals also interfere with enzyme systems leading to reduced productivity. Mitosis is generally inhibited by toxic metals, plasma membrane integrity is reduced, and ion uptake from the soil solution is compromised.

Aluminum

Aluminum (Al) is found in all soils and is often extremely toxic to plants at levels as low as 2–3 ppm at a pH of 5.5 or lower (Rout *et al*., 2001). Aluminum is especially toxic to seedlings and may be hard to identify. Plant symptoms resemble phosphorus deficiency and include: (i) general stunting; (ii) small, dark green leaves; (iii) purplish stems and leaves; and (iv) death of leaf tips. Sometimes symptoms are due to an aluminium-induced calcium deficiency which may include curling young leaves or death of growing points. In any case, the most widely seen effect of excessive aluminum is an inhibition of root growth, particularly an inhibition of cell division in root tips and lateral roots. New root growth is inhibited while lateral roots become thickened and brown. The root system forms many stubby roots and lacks branching.

The inhibition of cell division may be due to aluminum binding to the DNA molecules which increases the rigidity of the double helix which in turn decreases DNA replication. Aluminum also increases cell wall rigidity by cross-linking pectins, making the cell wall less flexible. In addition, aluminum causes a decrease in root respiration, interferes with sugar phosphorylation, and the deposition of polysaccharides in cell walls.

Aluminum also interferes with phosphorus nutrition by fixing phosphorus in an unavailable form in the soil or on the surface of plant roots. Aluminum also interferes with uptake, translocation and utilization of the essential nutrients phosphorus, calcium, potassium, magnesium, and iron. Some plants are tolerant of high levels of soil aluminum, attributed to uptake and utilization of calcium and phosphorus even in the presence of excessive aluminum. This tolerance seems to be controlled by a single gene in many species. Resistance to aluminum toxicity may result from a plant's ability to sequester excess aluminum by binding it to specific proteins.

The biochemical effects of aluminum on plants are associated with the alteration in structure and function of root cell membranes. Aluminum can bind to either the proteins or lipids in a membrane, depending on pH and other conditions, thereby reducing the membrane's fluidity. Aluminum causes phosphate anions to bind to the cell wall while preventing calcium from doing so.

Aluminum toxicity is also related to nitrogen metabolism. Nitrate (NO_3^-) uptake is reduced while ammonium uptake increases under high aluminum levels. Excessive aluminum can also interfere with the symbiotic relationship between *Rhizobium* and nitrogen-fixing species by reducing bacterial multiplication and nodule formation. NO_3^- uptake by soybean decreased when aluminum concentration in solution increased from 10 to 50 mM. Aluminum increased ammonium uptake and H^+ release in aluminum-sensitive sorghum cultivars.

At the subcellular level, aluminum interferes with ATP production by inhibiting ATPase activity. Drastic decreases in the ATP pool have been observed under high aluminum levels.

Arsenic

Arsenic is an extremely toxic element (Meharg and Hartley-Whitaker, 2002). It is often associated with mining and can be found contaminating groundwater, crops irrigated with contaminated water, and livestock which ingest contaminated feed and water. In addition, arsenic was an ingredient in many pesticides which lead to contamination of prime agricultural land.

Arsenic exists in the environment as inorganic arsenate, As(V), and arsenite, As(III), with both forms available to plants in the soil solution. Microbes in the soil, mammals, and invertebrates may metabolize inorganic arsenic to organic forms.

Arsenate is the predominant form of arsenic found in most soils and it competes with phosphate for uptake and utilization by the plant. Some species are resistant to arsenic and this resistance is generally due to reduced uptake and is controlled by a single gene. Reduced arsenic uptake can be induced by increasing the available phosphate in the soil solution. Even with resistance, species often accumulate appreciable levels of arsenic in their tissues. These species must compartmentalize or transform the arsenic to a less toxic form.

Arsenic toxicity to plants was first studied in the USA through pesticide residues in rice (*Oryza sativa*) grown on land previously devoted to cotton (*Gossypium* spp.) production. The pesticide monomethyarsonate (MMA) was used extensively in cotton production. Rice grown on old cotton land show symptoms of a disorder called straighthead disease, a disease associated with decreased flower fertility, which is caused by exposure to arsenic. Rice cultivars varied considerably in their susceptibility to arsenic toxicity.

Arsenic poisoning in plants causes symptoms ranging from reduced root growth to death. Arsenic competes with phosphate in the formation of ATP. Exposure to arsenic results in the production of ROS as arsenate is converted to arsenite. Additional ROS are generated when the arsenite is methylated.

As a major mechanism for arsenic tolerance, plants synthesize phytochelatins (PCs, [γ-glutamate-cysteine]n-glycine) when exposed to inorganic arsenic. The PC and arsenic form a complex rendering the arsenic non-toxic as long as the pH is acidic. The AS–PC complex is not stable at higher pH and arsenic can re-oxidize and become toxic.

Plants which grow on arsenic-contaminated soils are often mycorrhizal. Mycorrhizal fungi assist plants in acquiring phosphorus, thus it must be considered that the fungi might also increase arsenic uptake as well. Infection of both tolerant and intolerant plants by mycorrhizal fungi increases arsenic resistance.

Cadmium

Cadmium never exists as an isolated metal in nature, but rather as an integral part of lead:zinc mineralization. Cadmium finds its way into the environment as waste products from human industrial activity including power generation, metal working, electroplating, and the manufacturing and/or inappropriate disposal of nickel-cadmium batteries. It is a most dangerous metal: it is extremely soluble in water, extremely mobile and it takes very little cadmium to induce toxicity (Das *et al*., 1997).

Exact effects of cadmium on plants are not well understood, but cadmium can alter the uptake and utilization of other minerals (phosphorus, manganese, iron, calcium, magnesium, potassium) by interfering with them or through cadmium's deleterious effects on soil microbes that make nutrients available. Most of the cadmium absorbed by plants remains in the roots and is not translocated.

Some of the classic symptoms of cadmium poisoning in plants are leaf rolling, chlorosis, and general growth stunting. Cadmium has been shown to have many metabolic effects on plants including: (i) reduced absorption of nitrate; (ii) reduced nitrogen fixation in nodules of nitrogen-fixing species; (iii) decreased plasmalemma permeability and reduced water content of cells; (iv) decreased ATP formation; (v) enhanced lipid peroxidation and reduced membrane functionality; (vi) inhibited chlorophyll synthesis; and (vii) reduced carbon fixation. Cadmium probably causes these abnormalities by inducing free radical production and thereby inducing oxidative stress or by interfering with stress-coping mechanisms.

One mechanism plants utilize for minimizing cadmium damage is to sequester the ion in its roots via chelation by components of the cell wall. Another line of defense for the plant would be via immobilization by the plasma membrane. Cadmium could also be chelated by metallothioneins (MT) or PCs, both polypeptides, and stored in the vacuole, rendering it ineffective in causing damage.

Chromium

Chromium is widely used in industry, particularly the leather-processing industry. It is found in water, air and soil and is a serious environmental hazard to living organisms (Shanker *et al*., 2005). Hexavalent chromium (VI) is extremely toxic while trivalent chromium (III) is slightly less toxic. Hexavalent chromium usually occurs as chromate (CrO_4) or dichromate (Cr_2O_7) oxyanions and is a very potent oxidizing agent. Much of chromium's damage comes from its generation of ROS. Trivalent chromium is usually bound to organic matter and is therefore usually less toxic.

Chromium uptake by a plant is via uptake carriers for essential plant nutrients such as sulfur, iron, and phosphorus. Hexavalent chromium uptake by plants is an active process while uptake of trivalent chromium is passive. Movement is primarily in the xylem, although most absorbed chromium stays in the roots.

Seed germination is greatly reduced in the presence of chromium. Stem and root growth are also diminished on exposure to chromium. Leaf number may be only half of normal when a plant is exposed to chromium, and this leads to a great reduction in yield.

On a metabolic level, chromium can reduce photosynthesis as much as 95%! This is amazing since most of the chromium remains in the roots and the disruption occurs in the leaves. The inhibition of photosynthesis is due to disruption of the organization of chloroplast ultrastructure, inhibition of electron transport or by negative effects on the enzymes of the Benson-Calvin cycle. Other metabolic abnormalities like those described for the other heavy metals also occur with exposure to chromium.

Mercury

Mercury toxicity to plants arises from mercury pollution (Patra and Sharma, 2000). About two-thirds of the mercury pollution arises from natural causes while a third is generated by humans. In agricultural soils, much of the mercury comes from sludge, manures and fertilizers as well as seed-coating pesticides.

Even though mercury toxicity to plants is a problem, it is not that widespread, since mercury is not very available for plants to take up. Mercury tends to accumulate in the roots, thus any mercury found in the shoots comes from foliar absorption. With respect to foliar absorption, C3 species absorb five times more mercury than C4 plants. Most of the mercury in plants responsible for toxicity is from airborne sources.

Mercury may cause toxicity by altering membrane permeability, reacting with phosphate groups of ADP and ATP, thereby altering energy metabolism, or by replacing major cations in key metabolic processes. Mercury can replace magnesium in chlorophyll thereby inhibiting photosynthesis.

Transgenic plants capable of cleaving mercury ions from methyl-mercury complexes and then reducing them to the less toxic metallic form have been developed. Such plants which include *Arabidopsis thaliana, Brassica* (mustard), *N. tabacum* (tobacco) and *Liriodendron tulipifera* (tulip poplar) can be used in phytoremediation projects to remove mercurial contaminants from the soil.

Selenium

While some plants tolerate high levels of selenium in the soil and may be useful in phytoremediation of selenium-contaminated soil, most plants are selenium sensitive (Terry *et al*., 2000). Selenium has been considered by some a micronutrient and the range between sufficient and toxic is narrow.

Selenate is absorbed via active transport while selenite is absorbed passively. Plants can also absorb selenium from the air through their leaves.

When sensitive plants are exposed to excess selenium, symptoms of injury include: (i) stunted growth; (ii) chlorosis; (iii) withering of leaves; (iv) decreased protein synthesis; and (v) eventually premature death of the plant.

The major mechanism of selenium toxicity is through incorporation into proteins as selenium cysteine and selenium methionine in place of cysteine and methionine, respectively. This leads to altered form and function of the resultant proteins and an aberration in metabolism. In addition, selenium inhibits chlorophyll synthesis, nitrate reduction in leaves and inhibits the production of free radical scavengers.

Lead

Lead is a dangerous environmental pollutant, generally accumulating in the top layers of the soil (Sharma and Dubey, 2005). Most of our lead contamination comes from human activity: (i) lead from mining and smelting; (ii) lead-based paints and batteries; and (iii) leaded gasoline. Further contamination of agricultural lands comes from application of municipal sewage sludge as fertilizer. Some plants tolerate high levels of lead and/or accumulate high levels of lead in their roots. These plants can be used for phytoremediation of polluted sites.

Lead is easily absorbed by plants acting as a slow rather than acute poison. While high levels of lead can occur in both roots and shoots, lead tends to accumulate in the roots following absorption. Lead availability increases with pH between 3.0 and 8.5. Between pH 5.5 and 7.5 lead is generally not available for plant uptake due to precipitation with soil phosphates and carbonates. Lead in soils is usually tightly bound to colloids or organic matter. Furthermore, lead binds to mucilage on the soil roots preventing its uptake into the plant. Lead which makes it into the root is often bound to the cell wall. Some lead may be transported through the plasma membrane into the cell. Further transport through the root may be blocked by the endodermis. In general, tissues furthest away from the root are lowest in lead content.

The initial symptoms of lead toxicity are a rapid inhibition of root growth, general stunted plant growth, and chlorosis. Lead toxicity leads to many

physiological anomalies: (i) inhibition of sulfydryl-containing enzymes by alteration of tertiary structure; (ii) inhibition of seed germination; (iii) mitotic irregularities in root cells due to perturbation of microtubules; (iv) leakage of potassium from root cells, indicating interruption of membrane integrity; (v) reduced protein content and altered lipid content; and (vi) reduced synthesis of DNA and RNA. Lead also enhances the activity of certain enzymes, particularly those involved in hydrolytic activities and antioxidative enzymes.

Photosynthesis is greatly perturbed by lead toxicity. Chlorophyll, carotenoid and plastoquinone synthesis are inhibited and chloroplast ultrastructure and membrane composition is distorted. Enzymes of the Benson-Calvin cycle are inhibited as is electron transport. CO_2 deficiency occurs due to stomatal closure.

At high levels of lead exposure, respiration is decreased due to aberrations in electron flow in the electron transport system. At lower levels of exposure, respiration seems stimulated. RuBisCO is inhibited by lead exposure whereas oxygenase is unaffected. Thus under lead toxicity conditions, photosynthesis is inhibited while photorespiration is not. This might explain higher respiration and ATP levels under lead toxicity stress.

Lead generally blocks the uptake of potassium, calcium, magnesium, manganese, zinc, copper, iron, and nitrates into the roots by physically blocking sites of entry into the cells. Another mechanism for ion blocking by lead is enzyme and membrane changes caused directly by lead toxicity.

Transpiration declines with exposure to lead. Both leaves and guard cells are smaller in lead-stressed plants. Lead also induces an increase in abscisic acid, which causes stomatal closure.

Boron

Boron deficiency is more prevalent than an excess of boron. However, when it occurs, excessive boron can lead to many problems in plant production (Nable *et al*., 1997). Much of the boron in soil comes from the evaporation of seawater and much of that boron is in a fixed form unavailable for uptake by plants. Excessive levels of boron in the soil usually come from irrigation water that has a high boron content and occurs mostly in arid and semi-arid environments. Boron in the soil solution occurs primarily as boric acid, $B(OH)_3$, which is mobile in the soil and easily leached. Boric acid in the soil may also be adsorbed to organic matter or soil particles or absorbed by plants.

It is difficult to predict the potential for boron toxicity based on soil analysis since the total boron content may not reflect the plant-available boron. The highest boron concentrations in plant tissues tend to be in tissues at the end of the translocation stream, in particular, leaf tips and margins. Leaf analysis is also not a very good predictor of injury since different portions of the leaf may contain different levels of boron in them which may or may not indicate injurious levels. The range for plant-tissue boron levels that exhibit injury symptoms is often very wide.

General toxicity symptoms include leaf margin or tip burn in older leaves. In species where boron is phloem mobile, symptoms may appear in sinks such as fruits as gummosis or necrosis. Bark necrosis may also occur with excessive boron. Symptoms do not usually appear in roots. Other general symptoms of boron toxicity include: (i) decreased chlorophyll content; (ii) reduced growth; (iii) loss of leaf area; and (iv) leaf cupping.

Copper

While pH often regulates the amount of bioavailable metals in the soil solution, the amount of bioavailable copper is more closely related to the amount of organic matter in the soil than to pH (Reichman, 2002). Copper is strongly adsorbed to organic matter rendering it unavailable for plant uptake.

An initial symptom of copper toxicity is interveinal chlorosis in leaves. The initial chlorosis may be a reflection of iron deficiency induced by excess copper. The chlorosis often becomes cream colored or whitish. If the leaves also exhibit redness, there may be a copper-induced zinc deficiency as well. As the toxicity intensifies leaf tips and margins become necrotic. Eventually the entire leaf will become necrotic. In cases of acute toxicity, leaves often wilt quickly then become necrotic. In some species foliage becomes purplish. Copper toxicity also affects the roots, often long before shoot symptoms are visible. Root growth is often stunted and discolored and may be prone to fungal infection. Root hair production may also be inhibited (Reichman, 2002).

Manganese

Manganese becomes more soluble in the soil solution as pH is lowered making the possibility of

manganese toxicity more likely in acid soils (Reichman, 2002). Soils high in organic matter may generally exhibit less manganese toxicity than highly mineral soils. However, in soils high in other cations, manganese cannot readily form organic complexes, thus toxicity of manganese is more related to pH than to soil organic matter content. Waterlogged and compacted soils tend to exacerbate manganese toxicity problems by inducing the formation of the Mn^{2+} ion which is highly soluble and thus available for inducing toxicity.

Manganese toxicity is often characterized by brown spots on leaves, petioles and stems which ultimately become necrotic. Symptoms begin on older leaves and progress to younger ones eventually causing death if severe enough. Other symptoms may include general leaf bronzing or crinkling of leaf blades. Leaf crinkling may actually be manganese-induced calcium deficiency. Roots may be brownish and sometimes display cracking. Manganese toxicity may also induce iron or magnesium deficiencies (Reichman, 2002).

Zinc

Zinc is more soluble at a low pH making zinc toxicity a possibility in acid soils (Reichman, 2002). High levels of organic matter in soils helps form complexes with zinc making it unavailable for plant uptake, thus leading to reduced zinc toxicity.

Zinc toxicity is often characterized by a general chlorosis of younger leaves. Anthocyanin production may increase with zinc toxicity, thus leaves may become red. Zinc toxicity also leads to smaller leaves which may be vertically oriented. Leaves may also become necrotic. Root growth is reduced by zinc toxicity with a shorter primary root and fewer lateral roots which may become yellow. Zinc toxicity may also induce an iron deficiency and interfere with phosphorus metabolism in the plant (Reichman, 2002).

Alleviating soil mineral toxicity

In order to reduce economic and aesthetic damage caused by toxic mineral elements, a number of different strategies have been developed for alleviating soil mineral toxicity. With water-soluble toxins, high levels can sometimes be leached out of the soil via overhead irrigation. Another approach is to change the soil pH to change the toxin's solubility, forcing it to become adsorbed to soil particles or organic matter or to precipitate out. Another approach is to remove the toxin by growing tolerant species that absorb large quantities of the toxin and harvesting the plants. The problem though then becomes of what to do with the removed plant material. A final approach is to develop toxin-resistant cultivars that are able to produce acceptably on otherwise toxic soils. Resistant cultivars may exclude toxic metals from uptake, excrete excessive amounts of specific metals back into the soil, or sequester excess metals in the vacuole.

Soil salinity and sodicity

Around 6% of the earth's land is saline or sodic. Saline soils have enough soluble salts in them so that they can inhibit plant growth and development. Sodic soils are soils where the negative sites of clay particles are occupied by sodium ions. Salinity is measure by electrical conductivity (EC) and a saline soil is one with an EC of 4 dS/m or more. Many crops are affected by soils with an EC lower than 4 dS/m (Munns, 2012).

Soil salinity

There are two main types of salinity: (i) natural; and (ii) human induced. Natural salinity in soils is the result of either natural weathering of parent material which releases salts into the soil or deposition of salt from seawater by wind and rain. Salts of natural salinity include chlorides of sodium, calcium, and magnesium, as well as sulfates and carbonates. Sodium chloride is the principle salt from seawater.

While you might think that deposition from seawater is minimal, but it can actually be significant, especially at locations close to the water source (usually an ocean). Most wind/rain-deposited seawater has from 6 to 50 mg/kg of salt in it. If we use 10 mg/kg as a general average, 10 kg/ha of salt would be deposited for each 100 mm of rainfall/year. This salt accumulation is constant, so over time the salt added to the soil is significant. Clay soils would retain much of that salt while sandy soils would not, both retention rates depending on annual rainfall.

Human-induced salinity arises after land is cleared and planted with annual crops. Natural perennial vegetation often relies on a deep water table while annual crops usually require frequent irrigation in most parts of the world. The irrigation

of annual crops most often supplies excess water, thereby raising the water table and mobilizing salts deep in the subsoil. These salts are brought to the surface. Eventually, the soil becomes salty enough to be considered saline.

A second human-induced source of salinity is through irrigation which uses water rich in salts or is on insufficiently drained land. Even with good quality irrigation water ranging from only 200 to 500 mg/kg of soluble salt, irrigation of 1 ha with water of 500 mg/kg salt content, would deposit (assuming 6000–10,000 m^3 of water/ha/year) 3–5 t of salt. Crops remove very little of this salt, thus it will accumulate in the root zone. Eventually it will reach levels considered saline. Insufficiently drained soil does not allow excess salt, either natural or human deposited, to be leached from the soil and the soil is eventually considered saline.

Soil salinity is easily determined by measuring electrical conductivity (EC = dS/m). While we can measure a soil's EC, we also often measure the EC of irrigation water, rainwater and fertilizer solutions in hydroponic and greenhouse culture. Pay attention to the units used in reporting EC as it is often reported as deciSiemens per centimeter (dS/cm) (1000 dS/m) rather than deciSiemens per meter. Quality of water is often expressed as total soluble salts (TSS) with the international convention of 1 dS/m equal to 640 mg/l TSS (Munns, 2012).

On a large scale, soil salinity is often mapped using an electromagnetic (EM) meter which estimates the bulk EC of the soil. A transmitter coil sends out an impulse of alternating current which generates a primary magnetic field in the soil which causes a secondary magnetic field to develop which depends on soil conductivity. The primary and secondary magnetic fields are detected by a receiver and the readings generated into a reading of soil salinity.

Soil sodicity

Sodic soils are low in soluble salts but very high in exchangeable sodium (Na^+) which binds to negative charges on soil particles, particularly clay and organic matter. Sodium causes soil structure degradation. Soil sodicity is defined as an exchangeable sodium percentage (ESP) which imparts soil degradation. Sodic soils are also generally very alkaline with a pH of 8.5–10.

Normally, soil particles are held together by divalent cations such as Ca^{2+}. When a monovalent cation such as Na^+ replaces the divalent cation in low-soluble salt soils, the soil aggregates swell and clay particles separate from each other, thereby destroying the structure of the soil. Globally, soils are considered sodic if the ESP is from 6 to 15. The ESP that is considered sodic varies, since the destruction of soil structure depends on the soil's divalent cation content. In areas where the divalent cation content is naturally low, the ESP needed for damage is lower than regions with a higher divalent cation content.

Sodic soils develop over a long time. Naturally occurring salts or salts deposited by irrigation or rainwater eventually cause the clay particles to become saturated with Na^+. At the same time, irrigation often leaches divalent cations from the soil, exacerbating the situation. Eventually the clay particles that have dispersed due to the sodium begin to settle deeper in the soil profile and clog pores, impeding water drainage. The soil becomes poorly drained and waterlogged.

Salinity and plants

Soil salinity inhibits plant growth in two major ways: (i) osmotic stress; and (ii) direct salt injury (Munns, 2002; Munns and Tester, 2008). The decreased osmotic potential of the soil solution with a high concentration of salts induces osmotic stress. Plants have a difficult time absorbing adequate amounts of water from the soil. The water that is absorbed has high levels of sodium and chloride ions in it, which can directly injure cells, especially in leaves.

Species differ greatly in their level of salt tolerance. Barley (*H. vulgare*), for example, is quite salt tolerant while rice (*O. sativa*) is sensitive. Salt tolerance or sensitivity is determined based on the salt level needed to reduce biomass production or yield over time. The lower the level of salt needed for injury or decreased yield, the more sensitive the species.

Salt-induced osmotic stress results in reduced root and leaf growth as well as a decrease in stomatal conductivity, which in turn reduces photosynthesis. These responses and others resemble those observed during drought stress (see Chapter 8, this volume).

A direct effect of absorbed salt which leads to very high levels of Na^+ and Cl^- in leaves, especially older ones, is an increase in senescence of older leaves. Salt does not directly cause a reduction in the production of new leaves. If fewer new leaves

are produced relative to senescing leaves, the plant cannot photosynthesize enough to support a crop.

There is little genotypic variation in plant responses to salt-induced osmotic stress. Variation in resistance to direct salt injury is observed as differences in the ability to regulate the uptake of Na^+ and Cl^-. Salt-tolerant species take up little Na^+ and Cl^- while sensitive species absorb large quantities of Na^+ and Cl^-. Most crops can only tolerate a maximum of about 100 mM NaCl or 10 dS/m EC before a significant reduction in growth or yield occurs. In both sensitive and tolerant species, enzymes do not adapt to high salt levels in the cytoplasm, thus salt must be sequestered in the vacuole, away from enzymes.

Halophytes are a specific group of salt-tolerant plants. Their salt tolerance is not due to any special metabolic adaptations to high levels of Na^+, but rather their ability to compartmentalize very large amounts of Na^+ in vacuoles.

Alleviating stress

A number of approaches may be taken in an attempt to reclaim saline or sodic soils. In irrigated soils that have become saline or sodic, large quantities of water are applied at once in an attempt to remove the salt left behind by irrigation. This process called reclamation is slow, expensive, and requires good water drainage.

In sodic soils, sodium ions can be replaced with calcium ions by applying large quantities of gypsum (calcium sulfate) to the soil, followed by excessive water application to leach the sodium out of the soil. Gypsum is preferred to limestone as a calcium source since it has little effect on soil pH.

When saline or sodic soils interfere with seed germination and crop establishment, the top layer of soil is removed along with its excessive amounts of salt to enhance seed germination and seedling growth. Another approach is to irrigate with high quality water just prior to seeding, perhaps combined with ridges, to remove salts from the top layer of soil. Raised beds or ridges help minimize salinity effects. If ridging is not feasible, the entire field can be flooded prior to planting with good quality water. Just before all of the water has percolated into the soil, the seeds can be sown allowing them to settle into the mud by gravity.

Other management practices that can help alleviate problems associated with saline soils include: (i) mulching; (ii) deep tilling; and (iii) the incorporation of organic matter. Mulching reduces evaporation of water from the soil and its concomitant elevation of surface-soil salt levels. Additionally, mulching reduces irrigation frequency. Deep tilling involves mixing the surface layer of soil where much of the salt is into the deeper layer of soil, thereby diluting its concentration. Adding organic matter to the soil improves soil structure and drainage.

If alleviating the stress caused by salt or sodium in the soil is not possible, salt-tolerant species or cultivars can be utilized. Reclamation of abandoned salinized land with halophytes is useful in returning the land to productivity. Tallwheat grass (*Thinopyrum ponticum*) and saltbush (*Atriplex amnicola*) are halophytes that can be grown on salinized land as fodder. Once the salt has been removed from the soil, other less tolerant species can be re-introduced.

7 Water and Plants

Water is amazing and essential to life on earth. In this chapter, we will explore the many ways in which water is vital to plant life and how we use water as a management tool to improve productivity in many horticultural endeavors. A particularly nice reference on exploring water from many different angles is *Water Structure and Science* (Chaplin, 2011).

Basic Properties of Water in Plants

Water is a phenomenal substance. Without it, life as we know it would not exist. Some of the properties which make it so extraordinary include its specific heat, heat capacity, heat of fusion, heat of vaporization, and density (Table 7.1).

Specific heat

The specific heat is the amount of energy required to raise the temperature of 1 g of a substance by 1°C. It is an intensive variable meaning that it is a property that is independent of the quantity of the substance in question. The specific heat of water, higher than any other common substance, is 1 cal/g/°C. The specific heat of copper is 0.092 calorie/g/°C. So what? This means that it takes almost 11 times (1.0/0.092 = 10.87) as much energy to heat 1 g water from 20 to 21°C compared with heating a penny from 20 to 21°C. If water requires so much more energy for the same mass as copper, where does the energy go? It is stored as potential energy in the water. All substances have a certain amount of internal energy associated with them. This energy is a combination of kinetic (molecular movement) and potential (stored with a capacity to do work) energy. Temperature is a measure of kinetic energy, thus the difference in specific heat of two substances reflects the difference in their potential energy. The large amount of potential energy of stored in water makes anything containing water (i.e. plants) very resistant to sudden changes in the temperature of the surrounding environment.

Heat capacity

Heat capacity is different from specific heat capacity. Heat capacity is the amount of energy required to raise the temperature of a substance (or an object) by 1°C. It is an extensive variable since it relies on the quantity of the substance in question to determine its value. For example, the heat capacity of 1 kg of water is much greater than that of 1 g of water, even though the specific heat capacity is the same regardless of mass.

This is important in looking at plants and their resistance to temperature change, particularly when considering frost injury to sensitive species. Larger plants or tissues have a greater heat capacity then smaller plants or tissues, thus are more resistant to frost injury (this is discussed further in Chapter 9, this volume).

Heat of fusion

The heat of fusion of water is 80 cal/g and is the amount of energy liberated when water freezes. We can use this heat given off when water freezes to measure the lethal low temperature for a number of horticultural crops in low temperature stress studies, for example peaches (*Prunus persica*), cherries (*Prunus* spp.), and azaleas (*Rhododendron* spp.). We also use this heat when using overhead irrigation to protect crops from frost injury. (Note that it is the resistance to temperature change until a total phase transition that a mixture of ice and water has that really protects from a frost.)

Heat of vaporization

The heat of vaporization for water is 540 cal/g and is the amount of energy absorbed when water goes from a liquid to a gas. A tremendous amount of energy is removed from plant leaves as water evaporates from them during transpiration.

Table 7.1. Properties of water that make it important in horticulture.

Property	Value	Importance
Specific heat	1 cal/g/°C	Makes plants very resistant to sudden changes in the temperature of the surrounding environment
Heat capacity	Varies	Makes larger plants or tissues more resistant to frost injury
Heat of fusion	80 cal/g	Allows measurement of freezing point; frost protection using overhead irrigation
Heat of vaporization	540 cal/g	Provides cooling energy during transpiration
Density at 0°C		
Liquid	0.9998 g/cm^3	Allows ice to float and prevents bodies of water from freezing from the bottom up
Solid	0.931 g/cm^3	
Universal solvent		Provides medium for life as we know it
Hydroxyl bond		Absorbs infrared energy making water vapor a potent greenhouse gas

Density

Water is a very odd substance when it comes to density. The density of most solids is greater than their liquid counterparts. The density of water at 0°C is 0.9998 g/cm^3 while ice at the same temperature has a density of 0.931 g/cm^3, thus ice is less dense than liquid water and it floats. Another interesting fact about water is that it has a maximum density of 1 g/cm^3 at 3.98°C. Why is this important? As a body of water cools in response to cool air above it and approaches its freezing point, it becomes denser and when it reaches 3.98°C, it sinks and is replaced with warmer, less dense water from below. Once the entire body of water is at 3.98°C, it will continue to cool and the top layer will freeze when it reaches 0°C (we're not considering freezing point depression cause by any dissolved solutes in the water). Since the ice is less dense than liquid water, it floats. Thus the body of water freezes from the top down. If ice didn't float, lakes, rivers and bays would freeze from the bottom up and probably freeze solid. As it is, the floating ice offers a layer of insulation to the water below, thus bodies of water don't normally freeze solid, but rather only a few centimeters to a few meters deep.

Water in the Environment

Water in the atmosphere

Our atmosphere always has water in it. Rain and fog are important water sources for plants and melting snow is important for recharging groundwater stores or reservoirs for use during the growing season. The energy released or absorbed (fusion and vaporization) during phase transitions of water in our atmosphere are critically important in managing the energy balance of our atmosphere.

Much of the time atmospheric water is in the form of water vapor we don't see. We see products of condensation (clouds, fog, or rain) (Fig. 7.1) or freezing (snow or ice crystals) in our daily weather, but water vapor is essentially invisible. Water vapor is also the most potent greenhouse gas, even though we often hear more about CO_2 and methane (CH_4). Water vapor is such a powerful greenhouse gas because of its hydroxyl bond which absorbs a tremendous amount of infrared energy.

Water vapor content of the air is expressed in a number of different ways: (i) dew point; (ii) vapor

Fig. 7.1. Early morning ground fog in September (New Jersey, USA). Fog forms as the air temperature reaches the dew point just before sunrise after a long night of radiative cooling. Note the fog is close to the ground indicating that this air has cooled to the dew point while air at 4 or 5 m and higher has not and remains above the dew point.

pressure; (iii) specific humidity; (iv) absolute humidity; and (v) relative humidity (RH). The dew point is the temperature at which water vapor in cooling air at a constant barometric pressure will condense to liquid water called dew. At the dew point, the air is saturated with water vapor, meaning that at the given temperature, pressure, and volume, the air could hold no more water molecules.

Understanding water vapor in the atmosphere relies on understanding the concept of vapor pressure. Imagine that you have an open container of pure liquid water that you keep at a constant temperature. At any given time water molecules might be leaving the surface of the water and entering the surrounding air. Additionally water molecules will be returning to the liquid. This movement is based on diffusion and over time if the container is not closed, more molecules would leave than return and eventually your container would become empty due to evaporation. More molecules leave the liquid water than return under most conditions because there are more water molecules in the liquid water per unit volume (in general) than there are in the air above the container and the molecules will move from an area with more molecules per unit volume to an area with fewer molecules per unit volume.

Now imagine placing a lid on your container. This will restrict the movement of water molecules to the volume inside the closed container above the liquid water surface. When the number of molecules leaving the liquid is in equilibrium with the molecules returning to the liquid state, the pressure exerted is called the vapor pressure. Neither the volume of your container nor the surface area of your liquid will affect the vapor pressure. Increasing or decreasing the temperature will raise or lower the vapor pressure, respectively. Saturation vapor pressure is the vapor pressure of the water when the container's vapor volume is saturated with water molecules.

We need to know about vapor pressure because water will move from a plant, primarily from the leaf, into the atmosphere based upon vapor pressure gradients.

We measure and often report the water content of the atmosphere as humidity. There are three different measures of humidity: (i) absolute; (ii) specific; and (iii) RH. Absolute humidity is the mass of water vapor per unit volume of air, expressed in grams of water vapor per cubic meter of air. Specific humidity is the ratio of the density of the water vapor to the density of the air, generally expressed as grams of water vapor per kilogram of air. Specific humidity does not vary with temperature or pressure, since it is a weight-to-weight value, and weight is constant as temperature and/or pressure changes.

RH is the most commonly reported value of humidity. It is expressed as a percentage of actual vapor pressure in the air to the saturation vapor pressure. Since the saturation vapor pressure changes with temperature (Table 7.2), the RH of a parcel of air will change if the temperature changes, even though the absolute amount of water vapor in that parcel remains the same.

For example, if the vapor pressure of a parcel of air at 10°C is 5.0 mmHg, the RH of that parcel is (5.0/9.21) × 100 = 54.3%. If we increase the temperature of the same parcel of air to 15°C without adding or removing water vapor, the RH is (5.0/12.79) × 100 = 39.1%. Thus at 15°C, the air is only 39.1% saturated while at 10°C it is 54.3% saturated. As air heats up, it takes more water molecules to saturate it.

If we know the RH, air temperature, and leaf temperature, we can calculate vapor pressure gradients between leaves and the air to understand water movement. It is useful to understand and compare the movement of water under different situations, for example, water use of leaves of the same plant in the sun versus the shade.

Let's look at a quick example. Suppose we have a generic plant at an air temperature of 20°C and 43% RH, some leaves are in the sun and some of them are in the shade. The leaves in the shade have a temperature of 20°C and the leaves in the sun are 30°C (we'll pretend that we measured the leaf with an infrared thermometer or a thermocouple).

Table 7.2. The saturation vapor pressure (mmHg) of water from –10 to 40°C.

Temperature (°C)	Saturation vapor pressure (mmHg)
–10	2.15
0	4.58
5	6.58
10	9.21
15	12.79
20	17.54
25	23.76
30	31.8
40	55.3

From Table 7.2, the saturation vapor pressure at 20°C is 17.54 mmHg and at 30°C is 31.8 mmHg. If we assume that the RH inside the leaf is nearly 100% and we know that the RH of the air is 43% as defined, we can calculate the vapor pressure of water in: (i) the air; (ii) the leaf in the shade; and (iii) the leaf in the sun.

Atmospheric vapor pressure at 43% RH, 20°C:

$$43 = (X/17.54) \times 100$$

$$0.43 = X/17.54$$

$$0.43 \times 17.54 = X$$

$$7.54 = X$$

Internal shade leaf vapor pressure at 100% RH, 20°C:

$$100 = (X/17.54) \times 100$$

$$1.00 = X/17.54$$

$$1.00 \times 17.54 = X$$

$$17.54 = X$$

Internal sun leaf vapor pressure at 100% RH, 30°C:

$$100 = (X/31.80) \times 100$$

$$1.00 = X/31.80$$

$$1.00 \times 31.80 = X$$

$$31.80 = X$$

Water vapor always moves due to a gradient, from a higher vapor pressure to a lower vapor pressure. We will assume that stomatal resistance and boundary layer resistance to water vapor movement are negligible in this example. Therefore, if you compare the vapor pressure of the leaves and atmosphere you observe:

Shade leaf versus air = 17.54 versus 7.54 = gradient of 10 from leaf to air

Sun leaf versus air = 31.80 versus 7.54 = gradient of 24.26 from leaf to air

Thus in both cases, water vapor will move from the leaf to the air. This is not surprising at all, since we know leaves transpire and water normally moves in this direction. What is interesting is to compare the rate of water vapor movement from shade leaves into the air versus movement from sun leaves into the air:

Sun leaf to air gradient/shade leaf to air gradient = 24.26/10 = 2.426

This number means that leaves in the sun use nearly one and a half times more water than leaves in the shade at the same air temperature and RH! You might have guessed this general result using common sense but may not have known the magnitude of the difference in water use. Now you can prove the magnitude of the difference is as great as it is.

These vapor pressure gradients between internal leaf spaces and the atmosphere are the driving force for transpiration. This is the exit region for water from the plant, so what about the entrance region?

Water in the soil

Soil is a plant's major water source. Soil consists of solid, liquid and gaseous portions, all changing in the percentage of the soil volume they occupy as plants use water and it is replenished or not by precipitation or irrigation. Water in the soil is the primary source of water for plants. Some water can be absorbed by leaves of some species; however, most species absorb water from the soil. The liquid water in the soil is divided into four categories: (i) field capacity; (ii) gravitational water; (iii) capillary water; and (iv) bound water.

Field capacity is the maximum amount of water a soil can hold and only exists immediately after it rains or irrigation has occurred. Water quickly drains (assuming good drainage) from the soil due to the forces of gravity and is appropriately called gravitational water. The water that remains is adsorbed to soil particles creating a thin film around them and is called capillary water. Water that is permanently bound to soil particles and is not available to plants is called bound water. Most of the water available to plants is capillary water, which slowly becomes more difficult to extract from the soil as the soil dries until the point has been reached that all of the capillary water has been used and only bound water remains. As water drains from field capacity through gravitational action, it is available to plants, but generally this water is available for only a short time after saturation.

Water molecules are adsorbed to the soil particles and held in pore spaces, the smaller the pore, the more tightly the water is held. As a soil is depleted of its available water, the remaining water is held more tightly. We are often interested in

knowing how tightly the water is being held in the soil because this gives us an idea of how easily a plant might retrieve it and also often gives us an idea how much water is in the soil.

When all of the available water has been removed from the soil where plants could absorb it, the soil is said to have reached the permanent wilting point. At this point, if soil moisture is not replenished, plants will probably die.

Water is also present in the soil as vapor. The RH of the pore spaces in soil is above 99% even at the permanent wilting point. This water vapor is important for plant growth. Interestingly nearly 85% of the water a seed imbibes during germination in non-saturated soils is water vapor (Wuest, 2007). The idea that seeds need good contact with the soil for good germination is somewhat incorrect. In a soil near field capacity, only 10% of the seed's surface is actually in contact with liquid water. It is more important to ensure that soil moisture losses are minimized during germination by covering the seeds adequately with either soil, mulch or both.

Measuring soil moisture

Soil moisture content can be measured simply by squeezing a handful of soil tightly and observing the ease with which the resulting ball of soil yields to slight pressure. Soils low in water will not form a ball readily and soils high in water content will form a stiff ball which does not readily yield to pressure.

A more reliable method of measuring soil water content is to measure the energy by which water is held by soil particles using a tensiometer. The greater the energy holding the water to the soil particles, the less water there is in the soil available to plants. A tensiometer consists of a porous ceramic tip at the end of a long glass tube with a pressure gauge attached at the top. The porous tip is buried in the soil at a standard depth (10, 20, 40 cm, etc.) and the glass tube is filled with water. A dye is often added to the water to facilitate viewing of the water level in the tensiometer after its tube has been buried in the soil. The pressure gauge is attached to the top of the tube. As water is pulled out of the glass tube through the porous tip in response to decreasing soil moisture, the gauge measures the tension which develops in the tube. Since the porous tip is porous to both water and salts, the osmotic potential of the soil does not influence readings obtained. Tension is negative and measured in kilopascals (kPa).

We are interested in this energy status (water potential) of water in the soil because water always moves due to a gradient in energy, from a region of high energy to a region of low energy. Just remember that water always moves downhill. The greater the tension, the less available water there is for plants to utilize. Though tension measurements have a negative value, the negative sign is often omitted. If the water potential of the soil as measured by a tensiometer is –12 kPa and a plant root has a water potential of –16 kPa, water would move from the soil into the root. It's not exactly that simple, but it illustrates the need for understanding soil water potential.

Plants and Water Use

Most plants require a tremendous amount of water to survive. This water is needed as a major component of the plant tissues; up to 98% of a plant's fresh weight is water. Water is also needed to maintain a plant's turgor. Without adequate water, plants wilt. Water is also important for cooling leaves via transpiration and evaporation of water from leaves, which removes a tremendous amount of heat energy from the leaf in the process. Water is also a major metabolic player. It is the universal solvent and is involved in many metabolic processes.

The amount of transpiration in a plant varies greatly with species and the environment. As an example, 225 kg of water is used by a single corn plant to produce 1 kg of dry matter. Over its lifetime, a typical plant may use over 100 times its own fresh weight in water. A reasonable estimate of typical transpiration on a leaf area basis is 0.5–2.5 g water/cm^2/h. This translates into as much as 200 l/h for a 'typical' tree. One hectare of corn (*Zea mays*) may transpire as much as 0.6 million l of water in one growing season. This amount of water is equivalent to a 0.4 ha lake, 38 cm deep.

In modern horticulture we need to know how much water a plant needs to maximize quality and quantity of the commodity in question. If we know how much water a plant needs over a defined time frame, we can supply supplemental water via irrigation if needed.

Since exact amounts of water needed for many crops varies by cultivar, location, environmental conditions, soil qualities, etc., a much more useful tool would be one which allows us to estimate how

much water a particular plant or crop needs. Such a tool is an estimate of evapotranspiration, the combined loss of water from the soil via evaporation and transpiration from the plant.

Estimating Water Use by Plants

In order to know how much water is needed from irrigation, we must be able to estimate how much water a crop needs over time and compare that to actual precipitation over the same time period. By using mathematical equations and easily obtained data from local weather stations, we can estimate how much water is used in evapotranspiration and we call our estimate, reference evapotranspiration (RET, formerly called potential evapotranspiration). Simple estimates of RET can be derived from measurements of incoming solar radiation, wind, temperature, and RH. Water use will vary from plant to plant, crop to crop and from day to day. However, these estimates are meant to be calculated daily and summed over time, which provides a fairly good estimate of the amount of water needed.

The ASCE-EWRI Standardized Penman-Monteith equation

The ASCE-EWRI (American Society of Civil Engineers – Environmental and Water Resources Institute) Standardized Penman-Monteith equation is considered the standard equation for estimating the daily evapotranspiration from a crop (Monteith, 1965; Allen *et al*., 1998).

$$ET_{sz} = \frac{(0.408\,\Delta(R_n - G) + \lambda \frac{C_n}{T+273} U_2(e_s^o - e_s)}{\Delta + \lambda(1 + C_d U_2)}$$

Where:
ET_{sz} = the standardized evapotranspiration for a short (ET_{os}) or tall (ET_{rs}) reference crop in millimeters per day (mm/day)
Δ = the slope of the vapor pressure curve [kPa/°C]
R_n = net radiation at the crop surface [MJ/m^2/day]
G = soil heat flux density [MJ/m^2/day]
λ = latent heat of vaporization of water = 2.45 MJ/kg (which is a psychrometric constant)
C_n and C_d are reference values for specific crops: Cn = 900 and 1600 for short or tall crops, respectively, and Cd = 0.34 or 0.38 for short or tall crops, respectively.
T = mean air temperature in °C
U_2 = wind speed in meters per second (m/s) at 2 m above the ground
e_s^o = the saturation vapor pressure of water at T
e_a = the actual vapor pressure of water at T, derived from RH

Let's examine the calculations needed in order to estimate evapotranspiration from the following weather data: average daily (24 h average) values for solar radiation, wind, temperature, and RH. We will examine the calculations as we walk through the equation to see where we can derive the pieces of information needed to complete our estimate. Where possible, variables that can be generalized to a constant value for most locations and conditions will be utilized for our discussion. Those who wish to use a more rigorous approach with more calculations are encouraged to check the references.

$$ET_{sz} = \frac{(0.408\,\Delta(R_n - G) + \lambda \frac{C_n}{T+273} U_2(e_s^o - e_a)}{\Delta + \lambda(1 + C_d U_2)}$$ [AU 1]

Let's assume λ = 0.067 for an altitude of 0 m (sea level), making our equation:
(See equation (1) at bottom of page).

The value of Δ

We need to know the value of Δ which we can derive from the formula: [AU 1]

$$\Delta = \frac{4098\left[0.6018\,exp\left(\frac{17.27T}{T+237.3}\right)\right]}{(T+237.2)^2}$$

Where:
Δ = slope of saturation vapour pressure curve at air temperature T [kPa/°C]

$$ET_{sz} = \frac{(0.408\,\Delta(R_n - G) + 0.067 \frac{C_n}{T+273} U_2(e_s^o - e_a)}{\Delta + 0.067\,(1 + C_d U_2)} \quad (1)$$ [AU 1]

T = air temperature [°C]
exp[..] = 2.7183 (base of natural logarithm) raised to the power [..]

R_n: Net solar radiation at crop level

Let's take a look at R_n, the net radiation at the crop surface [MJ/m²/day], which normally turns out to be positive except under extreme conditions. R_n is the difference between the incoming net shortwave radiation (R_{ns}) and the outgoing net longwave radiation (R_{nl}):

$$R_n = R_{ns} - R_{nl}$$

We need estimates for R_{ns} and R_{nl}.

R_{ns} is the net shortwave radiation resulting from a balance between incoming and reflected radiation, given by the equation:

$$R_{ns} = (1 - \alpha)\, R_s$$

Where:
R_{ns} = net solar or shortwave radiation [MJ/m²/day]
α = albedo or canopy reflection coefficient, which is often estimated as 0.23
R_s = the incoming solar radiation [MJ/m²/day]

To complete the above calculation, we need an estimate of R_s. We can get that from the following formula:

$$R_s = \left(0.25 + 0.75\frac{n}{N}\right) R_a$$

Where:
R_s = solar or shortwave radiation [MJ/m²/day]
n = actual duration of sunshine in hours [h]
N = maximum possible duration of sunshine or daylight hours [h], calculated as:

$$N = \frac{24}{\pi}\omega_s$$

Where:
ω_s = sunset hour angle [rad], which is:

$$\omega_s = arccos\ [-\ tan\ (\varphi)\ tan\ (\delta)]$$

φ = latitude [rad], which is:

$$[\text{Radians}] = \frac{\pi}{180}[\text{decimal degrees}]$$

R_a = extraterrestrial radiation [MJ/m²/day]
But we need to know R_a in order to finish the previous equation. We can calculate R_a from the following formula:

$$R_a = \frac{24(60)}{\pi} G_{sc} d_r [\omega_s sin_\varphi sin_\delta + cos_\varphi cos_\delta sin_{\omega s}]$$

Where:
R_a = extraterrestrial radiation [MJ/m²/day]
G_{sc} = solar constant = 0.0820 MJ/m²/min
d_r = inverse relative distance earth–sun, which is:

$$d_r = 1 + 0.033cos\left(\frac{2\pi}{365} J\right)$$

Where:
J = Julian date
ω_s = sunset hour angle [rad], which is:

$$\omega_s = arccos\ [-\ tan\ (\varphi)\ tan\ (\delta)]$$

φ = latitude [rad], which is:

$$[\text{Radians}] = \frac{\pi}{180}[\text{decimal degree}]$$

If you know your latitude in degrees and minutes, convert it to decimal degrees by the formula:

$$\text{decimal degree} = \text{degree} + \frac{\text{min}}{60}$$

δ = solar declination [rad], which is:

$$\delta = 0.409sin\left(\frac{2\pi}{365} J - 1.39\right)$$

Where:
J = Julian date
An example:
What is the extraterrestrial radiation (R_a) for a location 20°S latitude on 3 September?

$$R_a = \frac{24(60)}{\pi} G_{sc} d_r [\omega_s sin_\varphi sin_\delta + cos_\varphi cos_\delta sin_{\omega s}]$$

G_{sc} = solar constant = 0.0820 MJ/m²/min

(See equation (1) at bottom of page).

$$R_a = \frac{24(60)}{\pi}\ (0.0820)\ d_r [\omega_s sin_\varphi sin_\delta + cos_\varphi cos_\delta sin_{\omega s}] \qquad (1)$$

d_r = inverse relative distance earth–sun, which is:

$$d_r = 1 + 0.033cos\left(\frac{2\pi}{365}J\right)$$

Where: J = Julian date =246

$$d_r = 1 + 0.033cos\left(\frac{2\pi}{365}246\right)$$

$$d_r = 0.985$$

ω_s = sunset hour angle [rad], which is:

$$\omega_s = arccos\ [-\ tan\ (\varphi)\ tan\ (\delta)]$$

$$\omega_s = 1.527$$

φ = latitude [rad], which is:

$$[\text{Radians}] = \frac{\pi}{180}[\text{decimal degrees}]$$

$$[\text{Radians}] = \frac{\pi}{180}[-20] = -0.35$$

δ = solar declination [rad], which is:

$$\delta = 0.409sin\left(\frac{2\pi}{365}J - 1.39\right)$$

$$\delta = 0.409sin\left(\frac{2\pi}{365}246 - 1.39\right)$$

$$\delta = 0.120$$

Thus:

(See equation (1) at bottom of page).

Now we can calculate R_s:

$$R_s = \left(0.25 + 0.75\frac{12}{12}\right)R_a$$

$$R_s = (1)32.2$$

$$R_s = 32.2\,\text{MJ/m}^2\text{/day}$$

This calculation assumed a mostly sunny day where the total number of sunlight hours (n) was 12 and the total possible number of sunlight hours (N) was 12.

$$R_{ns} = (1 - \alpha)R_s$$

$$R_{ns} = (1 - 0.23)32.2$$

$$R_{ns} = (0.77)23.2$$

$$R_{ns} = 24.794$$

Remember, we are ultimately trying to calculate $R_n = R_{ns} - R_{nl}$, so we need an estimate of R_{nl}.

R_{nl}: Net longwave radiation

(See equation (2) at bottom of page).

Where:

R_{nl} = net outgoing longwave radiation [MJ/m^2/day]

σ = Stefan-Boltzmann constant [4.903 10^{-9} MJ/K^4/m^2/day]

$T_{max,K}$ = maximum absolute temperature during the 24 h period [K = °C + 273.16]

$T_{min,K}$ = minimum absolute temperature during the 24 h period [K = °C + 273.16]

e_a = actual vapour pressure [kPa]

R_s/R_{so} = relative shortwave radiation (limited to ≤ 1.0)

R_s = measured or calculated solar radiation [MJ/m^2/day]

R_{so} = clear-sky radiation [MJ/m^2/day] calculated as:

$$R_{so} = (0.75 + 0.00002z)R_a$$

Where:

z = elevation above sea level (m)

For our example, the minimum and maximum temperature for the day were 21°C and 27°C, the elevation is 0 m (we're at sea level) and the actual vapor pressure was 2.1 kPa:

$$R_{nl} = 0.000004903\left[\frac{300.16^4 + 294.16^4}{2}\right]\left(0.34 - 0.14\sqrt{2.1}\right)\left[1.35\frac{32.2}{24.15} - 0.35\right]$$

$$R_a = \frac{24(60)}{\pi}(0.0820)(0.985)[1.527(-0.041) + 0.93sin(1.527)]$$

$$R_a = 32.2\,\text{MJ/M}^2\text{/day} \quad (1)$$

$$R_{nl} = \sigma\left[\frac{T_{max,K}^4 + T_{min,K}^4}{2}\right]\left(0.34 - 0.14\sqrt{e_a}\right)\left[1.35\frac{R_s}{R_{so}} - 0.35\right] \quad (2)$$

R_{nl} = ?

So now we can calculate:

$R_n = R_{ns} - R_{nl}$

R_n = 24.794 – ?

Soil heat flux (G)

$$G = c_s \frac{T_i + T_{i-1}}{\Delta t} \Delta z$$

Where:
G = soil heat flux [MJ/m^2/day]
c_s = soil heat capacity [MJ/m^3/°C]
T_i = air temperature at time i [°C]
T_{i-1} = air temperature at time i–1 [°C]
Δt = length of time interval [day]
[AU 1] Δz = effective soil depth [m]

However, when values are calculated daily, G can be ignored, since the soil heat flux is going to be very, very small and thus is close to 0.

Wind speed

Wind speed is accurately measured with an anemometer in meters per second and expressed as: U_2 = m/s, measured 2 m above the ground.

$e_s - e_a$: The vapor pressure defecit

If we know the RH we can calculate e_a. Since RH varies over the day, we need an average RH value for the day (less desirable) or an average e_a value derived from RH_{max} and RH_{min} values for the day (more desirable). We must first retrieve values of e_s^o from a reference book, or we can calculate it if we know the air temperature using the following formula:

$$e_s^o = 0.6108 exp\left[\frac{17.27T}{T + 237.3}\right]$$

Where:
e_s^o = saturation vapor pressure at the air temperature T [kPa]
T = air temperature [°C]
$exp[\ .\ .\ ]$ = 2.7183 (base of natural logarithm) raised to the power [. .]

Remember to calculate an e_s^o value for both the T_{max} and T_{min}.
Now if we know $e^o_{s\,min}$, $e^o_{s\,max}$, RH_{max} and RH_{min}, we can calculate e_a from the formula:

$$e_a = \frac{e_s^o(T_{\min})\frac{RH_{max}}{100} + e_s^o(T_{\max})\frac{RH_{min}}{100}}{2}$$

From all of this, we want to calculate a vapor pressure deficit using the following formula:

$$e_s - e_a = \left[\frac{e_s^o(T_{max}) + e_s^o(T_{min})}{2}\right] - e_a$$

Estimated evapotranspiration

We now have all the information we would need to calculate an estimate of the evapotranspiration that would occur in 1 day.

$$ET_{sz} = \frac{(0.408\Delta(R_n - G) + \lambda\frac{C_n}{T+273}U_2(e_s^o - e_a)}{\Delta + \lambda(1 + C_d U_2)}$$

Where:
Δ = the slope of the vapor pressure curve [kPa/°C]
R_n = net radiation at the crop surface [MJ/m^2/day]
G = soil heat flux density [MJ/m^2/day]
λ = latent heat of vaporization of water = 2.45 MJ/kg (which is a psychrometric constant)
C_n = 1600
C_d = 0.38
T = mean air temperature in °C
U_2 = wind speed in meters per second (m/s) at 2 m above the ground
e_s^o = the saturation vapor pressure of water at T
e_a = the actual vapor pressure of water at T, derived from RH

With careful calculations and a bit of work, one can estimate the general water requirements for a crop and monitor precipitation to determine when, and if, and how much supplemental moisture is needed. For an excellent step-by-step walk through an example please visit http://edis.ifas.ufl.edu/ae459 or refer to Zotarelli *et al.* (2010).

Transpiration – Water Potential and Water Movement in Plants

Transpiration is especially important for cooling plant leaves, particularly those exposed to direct incoming solar radiation. It is also important for transporting nutrients from the soil to growing leaves and meristems and for providing water for the mass flow of nutrients from sources of photosynthates (leaves) to sinks (fruits, roots, meristems).

The driving force of transpiration is the difference in water potential between two components in the transpiration stream, such as soil to root, root to stem, stem to leaf, and leaf to air. Water potential is one of those concepts in plant physiology that many find difficult to comprehend, probably because they make it more difficult than it really is.

Water potential in plants: $\psi = \psi_{osm} + \psi_{pres}$

Water potential in plants consists of two major components, osmotic potential and pressure potential. Osmotic potential is that part of water potential regulated by the presence of molecules other than water in a solution. Those other molecules might include proteins, sugars or salts. Pressure potential is that component of water potential due to the increase in pressure within a cell due to the presence of a cell wall or the tension (negative pressure) generated by the pull of water molecules along the transpiration stream.

We indicate water potential symbolically as ψ. We indicate the osmotic component as ψ_{osm} and the pressure component as ψ_{pres}. Collectively they provide the following equation to describe water potential:

$$\psi = \psi_{osm} + \psi_{pres}$$

So what is the unit of measure? Osmotic potential and its components are measured in megapascals (MPa). Most of the time ψ_{osm} is negative (we'll see why shortly), ψ_{pres} is positive (unless under tension in the xylem where it would be negative) and the two components added together usually give ψ a negative value. Just how negative ψ is comparing one part of the transpiration stream to another determines the direction of water flow in the system.

In order to understand how the components of ψ influence water movement in the plant system, the energy concept surrounding ψ must make sense. A measure of molecular energy called Gibbs free energy is defined for pure water to be equal to 0.0. This free energy is the energy we call ψ. With this in mind, consider a container of pure water with molecules bouncing around inside the container. The energy contained in their bouncing around is the Gibbs free energy and is equal to 0.0. It seems a little strange that bouncing molecules have an energy value of 0.0, but that's the way it is.

If we now add some molecules of a solute such as glucose, sodium chloride, or a water-soluble protein, these solute molecules are going to interfere with the movement of the water molecules and slow them down. The free energy of the water molecules will be less than what it was with no solute, thus it will be negative. We would then say that the osmotic portion (ψ_{osm}) of ψ is <0.0. With no lid on the container, ψ_{pres} would remain 0.0. Thus $\psi = \psi_{osm} + \psi_{pres}$ will be negative. The more solvent added to the water, the more molecules interfere with water molecule movement, thus the more negative ψ_{osm} will be.

The ψ gradient

The same general scenario occurs in a plant cell except for the pressure potential. Since the cell is surrounded by a cell wall, pressure can build up and ψ_{pres} will be >0.0. In many instances, $\psi_{pres} < \psi_{osm}$, so ψ <0.0. In some cells, such as xylem cells, tension (ψ_{pres} <0.0) can develop which causes ψ to be very negative.

Water molecules move due to the gradient in ψ, moving from an area of higher (more positive) ψ to an area of lower (more negative) ψ. Water always moves downhill. The solute molecules may or may not move with the water, depending on barriers, such as cell walls and plasma membranes that separate the two regions in question.

Consider the general path of water movement from the soil through the plant into the atmosphere. Each component of the path has a ψ value depending on solutes and barriers which might generate pressure. Water will move along the pathway in response to differences in ψ. Table 7.3 includes hypothetical values for components in the pathway of water movement in a typical plant. Most living plant cells have an osmotic potential ranging from –0.5 to –1.2 MPa but may be as high as –2.5 MPa in cells storing a large amount of sucrose. Intercellular spaces in many roots and stems are typically around –0.1 MPa. Larger intercellular spaces in leaves may often reach –8.0 MPa. Pressure potential in typical cells ranges from 0.1 to 1 MPa but can be negative (about –1.0 MPa) in xylem cells. Typical soil solutions have a water potential between –0.1 MPa (soil at field capacity, low solute levels) to –2.0 MPa (significant water stress). During a drought, soils may reach a water potential of –2.5 MPa.

Looking at the values of ψ in Table 7.3, it is fairly easy to understand the movement of water through a plant due to differences in ψ. But there are other important forces at work which contribute to the mechanisms of transpiration.

Table 7.3. Hypothetical values of water potential ψ for various components in the path of water movement in a typical plant.

Component	ψ MPa
Soil solution	–2.0
Root epidermal cell	–0.025
Root endodermal cell	–0.1
Root xylem cell	–0.4
Stem xylem cell	–0.8
Leaf xylem cell	–1.2
Leaf mesophyll cell	–1.7
Intercellular space in leaf mesophyll	–8.0
Atmosphere surrounding leaf (20°C, 40% RH)	–100.0

ψ At the cellular level

The fact that a cell has a cell wall affects ψ tremendously by its influence on ψ_{pres}. Consider a flaccid root epidermal cell. There is a certain amount of solute in it and it will eventually fill a finite volume once it absorbs water. If we assume the number of solute molecules is not going to change all that much, we can assume that water will flow into the cell from the outside, thereby changing the concentration of molecules and ψ by changing ψ_{osm}. Once the volume is occupied, ψ will change due to changes in ψ_{pres} rather than ψ_{osm}. Once the volume of the cell is filled with water, not many water molecules can continue to enter, but each one that does, increases the pressure a great deal.

Equilibrium is often discussed when studying water movement in response to differences in ψ. However, equilibrium is never really reached since organisms usually exist in a system of gradients rather than any type of equilibrium. The gradients allow for the flow of the components of life among cells, tissue, organs and their respective environments.

Two adjacent cells will exchange water based on the difference in ψ between the two cells. Water will move from the cell with the more positive value of ψ to the cell with the more negative ψ. This movement will continue until the two cells have the same ψ. We might be tempted to say they are now at equilibrium. But even though ψ may be at equilibrium, this equilibrium was reached by changes in the ψ, and probably changes in the concentration of sucrose, sodium chloride, soluble proteins, etc. These concentrations are not in equilibrium, and since the plasma membrane is permeable, molecules of these solutes are likely to move across the membranes, altering each cell's ψ, causing water molecules to move, which causes changes in solute concentration, etc. So see how equilibrium is never really reached?

ψ Across the root

Let's take this discussion even further. Consider the movement of water from the soil across a typical root to the xylem for transport to the leaves. To reach the xylem cells, water has to traverse the root epidermis, the cortex, the endodermis, and finally the xylem cell wall. Water moves across the root symplastically from the cytoplasm of one cell to the cytoplasm of an adjacent cell. As long as cells remain intact, there are no barriers to symplastic movement of water in plant roots. Water may also move apoplastically across the root, remaining outside of the cytoplasm, moving through intercellular spaces and cell walls. There is a barrier to water movement through the apoplast in a root and it is called the endodermis. It is a ring of suberized cell walls (casparian strips) surrounding the vascular cylinder, preventing apoplastic movement of materials into the xylem. In order for water or any solute to move into the xylem, it must do so symplastically. Since the plasmalemma is selectively permeable, some solutes can pass through while others cannot. The endodermis creates a barrier which helps regulate the movement of solutes into the xylem for transport to other regions of the plant.

A gradient in ψ_{osm} is maintained from the vascular cylinder across the root to the epidermis by a concomitant gradient in sucrose delivered from the leaves through the phloem. As sucrose diffuses into cells it can: (i) remain in solution affecting ψ_{osm}; (ii) be metabolized for root cellular processes; or (iii) it can be stored as starch which does not affect ψ_{osm} (starch is not soluble in water).

In addition, a gradient in ψ_{pres} exists across the root, and we should pay particular attention to the negative ψ_{pres} (or tension) which exists in xylem cells. This negative ψ_{pres} helps pull water into the xylem cells for transport to the leaves. This pull is made possible by water's cohesive properties. Cohesion is an attraction of like molecules, such as water for water, which keeps them held together tightly. Thus they in effect pull each other along. Another property of water, adhesion, is the attraction of water molecules for negatively charged surfaces. Glass is a good example of a negatively charged

surface. The xylem cell wall is also negatively charged. Thus water molecules are attracted to the xylem cell wall and to each other. These combined properties allow for capillary movement in water through the xylem vessels.

So we've moved the water from the soil across the root and into the xylem. Most of the water absorption in roots takes place in the root tissue just behind the root tip and the zone of elongation. The epidermis of older root tissue is usually too suberized to allow water entry.

The accumulation of minerals due to active transport across the endodermis into the vascular cylinder (stele) creates a rather negative ψ_{osm} which results in water movement into the stele. Under periods of low transpiration (night time, cloudy, cool weather) pressure may build up in the stele forcing water up through the xylem, often exiting the leaf via hydathodes, specialized structures at the margins of the leaves (Fig. 7.2). Droplets of xylem sap may appear along the leaf margin at the hydathodes. This is called guttation.

ψ Up the stem

Water movement up the xylem is possible due to capillarity of the xylem–water system, and the cohesive and adhesive properties of water. In order for the water to be pulled through the xylem, a continuous column of water must exist from the root through to the leaf. If this column breaks, it is called cavitation. Ultimately this break may repair itself and transpiration can continue. (Remember you have hundreds or thousands of these continuous xylem vessels in a given plant, thus if some of them cavitate, it's not the end of the world.) Some species have regulatory pits in xylem cells called bordered pits which can control the changes in pressure (tension) which causes cavitation, by controlling water movement in and out of the cell through the pits. Cavitation is then prevented for the most part.

Fig. 7.2. Guttation visible on leaf margins of kale (*Brassica oleracea*, Acephala Group) 'Red Russian' early in the morning.

The ultimate driving force for transpiration is the evaporation of water from the leaf. This is accomplished by the tremendous gradient of water potential from the leaf (–2 KPa) to the atmosphere (–100 KPa, 20°C, 50% RH). Liquid water bathes the cell walls of mesophyll cells inside the leaf, causing the water content of the intercellular spaces in the leaf to be at or near saturation. Water vapor then moves out of the leaf through the stomata where it evaporates into the atmosphere. This evaporation is the force which initiates the pull of water up through the xylem from the soil.

Environmental Factors Affecting Transpiration

The rate of transpiration in plants varies considerably among species and is affected by many environmental factors, especially light, temperature, RH, wind, and water (Fig. 7.3).

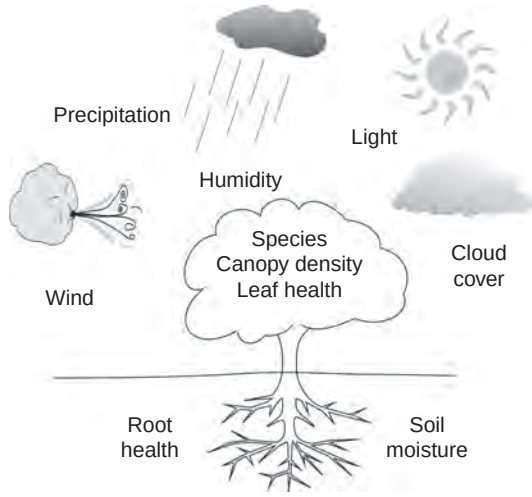

Fig. 7.3. Factors affecting transpiration. (Cloud, wind, precipitation and sun symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science, ian.umces.edu/symbols/.)

Light

Light regulates transpiration directly through effects on: (i) stomatal opening and closing; (ii) photosynthetic rate; and (iii) leaf temperature.

Stomata open and close in response to K^+ ions moving into and out of the guard cells and concomitant influxes and outfluxes of water. Blue light is absorbed by the pigment phototropin which activates a proton pump, driven by ATP from photosynthesis, which pumps H^+ ions out of guard cells. As protons are pumped out of the guard cells, K^+ enter the guard cells to relieve the increasing negative charge which develops in the guard cell due to H^+ leaving. This makes the ψ more negative, causing water to enter guard cells, increasing turgor and causing the stomata to open.

If soil water is not sufficient to keep up with transpiration requirements, the stomata must close. In most higher plants, abscisic acid (ABA) triggers stomatal closing. This often occurs around mid-day on a hot summer day. How does ABA do it? ABA binds to receptors on the surface of guard-cell plasma membranes which causes an increase in cytosolic pH and a transfer of Ca^{2+} from the vacuole to the cytosol. This stimulates a loss of anions, especially $NO3^-$ and Cl^-, as well as K^+ from the cell which reduces ψ and leads to a loss of turgor. The guard cells become flaccid and the stomata close.

Temperature

Transpiration increases with temperature for several reasons. Water evaporates faster at warmer temperatures. Additionally, metabolism increases (up to a point) with temperature, thus metabolic demands for water increase with temperature. In addition, when the temperature of a parcel of air increases, its RH decreases. This thereby increases the leaf-to-atmosphere water gradient, increasing transpiration.

Humidity

Transpiration increases with decreased RH. As the RH of the air decreases, the water vapor gradient between the leaf and the atmosphere increases, increasing the rate of transpiration. Even at 90% RH, the ψ of the atmosphere is much more negative (–14 KPa, 20°C) than the ψ of any leaf under any condition.

Wind

Wind primarily affects transpiration by influencing the depth of the boundary layer between a leaf and the atmosphere. The thin layer of air surrounding a leaf is called the boundary layer. The humidity of this boundary layer is nearly 100%, thus the thicker the boundary layer, the lower the rate of transpiration. On a calm day with little or no wind, this boundary layer can become relatively thick. On a windy day, this layer of moist air is constantly being swept away, thereby increasing transpiration.

Wind may also cool the leaf, resulting in reduced transpiration. Additionally, if it is windy enough, stomata may close, reducing transpiration.

Soil water

Two attributes of soil moisture affect transpiration. First, when water is plentiful in the soil, ψ will increase allowing more water to enter the root more quickly. As the soil dries, water is held more tightly by soil particles, decreasing ψ, making it more difficult for water to enter the root. The quality of the soil water also influences transpiration. If the soil water is salty due to natural reasons or excessive fertilizer applications, the ψ of the soil water will be more negative, regardless of the amount of water present, and this will make it more difficult for plants to access water, thus reducing transpiration.

Plant adaptations affecting transpiration

Many plant factors influence how adapted a plant is to reduced water availability. Some of these attributes may change as a plant develops throughout the growing season, others are genetically predetermined.

Larger leaves generally transpire more than smaller leaves. Leaf size may decrease as water stress occurs, reducing transpiration. Some plants adapted to regions with low available soil moisture inherently have small leaves.

Plants with a greater number of leaves tend to transpire more than those with fewer leaves. One major response of plants to water stress in an attempt to reduce transpiration and conserve water is a reduction in the number of leaves per plant via premature abscission. Wilting of leaves due to lack of turgor, reduces exposure to radiant energy,

thereby cooling the leaf somewhat and reducing photosynthesis and transpiration.

The number, distribution, and position of stomata greatly influence transpiration rate. Most stomata are on the underside of the leaf (abaxial). Fewer stomata per unit leaf area usually translate to reduced transpiration rates. Stomata may also be sunken, creating a miniature boundary layer immediately surrounding the sunken stomata which greatly reduces transpiration.

The cuticle thickness of a leaf influences the rate of transpiration. The cuticle is a waxy layer on all above-ground plant tissue which helps prevent direct water loss through epidermal cells. With a waxy covering in place, water is forced to move through the transpiration stream and out through the stomata. Thicker leaf cuticles reduce transpiration. Generally, plants from hot climates have a thicker cuticle than those from a cooler climate. Leaves growing in the sun also have a tendency to have thicker cuticles than those growing in the shade.

Leaf pubescence (hairiness) can alter transpiration. Epidermal hairs increase the boundary layer of a leaf, thereby reducing transpiration.

Antitranspirants

Antitranspirants are chemicals sprayed on plant leaves in an attempt to reduce water use by reducing transpiration. They act by either closing the stomata or coating leaves and clogging stomata to reduce transpiration. In general, the use of antitranspirants is not recommended as a common horticultural practice.

One exception to this rule is the use of an antitranspirant on evergreen species during the winter if they are exposed to harsh, drying winds and frozen soil. Frozen soil prevents water absorption and can even draw water out of the roots while the drying winds disturbs the boundary layer around leaves or needles, causing excessive water loss. In this situation, an antitranspirant may be of value for reducing loss from desiccation.

Plant Growth and Water Supply

Most plants require a steady supply of water during their growth and development for maximum production and best quality. Any interruption in the water supply will likely reduce final yields and crop quality. There are several crucial periods when lack of water can be extremely detrimental to production.

Any interruption of the water supply during germination could lead to death of the seedling. Roots are not usually extensively developed in the soil profile as the seedling germinates which makes a reliable water supply crucial. Later in the plant's development, roots may be developed enough to obtain water from deeper in the soil.

Rapid, early vegetative development is often necessary in many crops to establish a good plant framework on which to produce the crop. If vegetative growth is stunted, yields will likely be reduced. Once a good framework has been established, the next crucial stage is flowering.

Any water stress during flowering is likely to inhibit pollination and/or fertilization leading to flower abscission and lack of fruit. Stress after fertilization could lead to embryo abortion, and in most cases premature fruit abscission, greatly reducing yield.

In many fruit crops, water is crucial to the final stage of fruit development. That stage, final fruit swell, is a period of rapid and extensive cell enlargement. Any water stress reduces cell enlargement reducing final fruit size (an important economic consideration in many crops) and yield.

While water stress is usually detrimental to plant growth and development, there are times when stress from lack of water might lead to positive outcomes. In some crops, flowering or flower bud formation may be induced by lack of water. In protected culture of low-chill blueberries, water stress can substitute for the chilling required to break endodormancy. It is much easier to carefully induce water stress than to supply artificial chilling to plants in order to break dormancy and initiate out-of-season production.

Late-season irrigation should be monitored so that it does not induce growth. Late-season growth is often less cold hardy than early and mid-season growth. Slight water stress late in the growing season can sometimes increase the cold hardiness of some perennial species.

Irrigation – Supplying Extra Water

Agricultural irrigation is the number one use of water on a global scale. Water is becoming an increasingly precious commodity and effective management in horticultural production systems is imperative. As food for thought (no pun intended) it requires 2400 l of water to produce one average

hamburger, 200 l to produce one glass of milk, 135 l for one egg, 70 l for an apple, 40 l for a slice of bread, and 25 l for one average potato (FAO, 2011). Keep this in mind the next time you turn on the faucet (tap), go to the well, or fire up your irrigation pump.

The simplest irrigation system is a watering can or a garden hose with a sprinkler attachment. Most production systems, however, require a more elaborate set up. The major types of irrigation systems include ditch, overhead, or trickle, with their water source being groundwater, surface water, or a municipal water supply.

When considering a water source for irrigation, several components of irrigation must be considered: (i) water quantity needed; (ii) water sources and supply rate; and (iii) water quality.

Irrigation water

Water quantity

Any irrigation system must be able to supply a crop's water needs that are not met by the expected effective rainfall. Effective rainfall is that precipitation that falls and is readily absorbed by the soil. Ineffective rainfall is that rainfall that runs off due to intensity of precipitation or topography. In addition to the total amount needed over the growing season, consideration must be given for daily needs which will change over the crop's course of development. Water use is most often measured as: (i) inches of rainfall (or irrigation); (ii) acre-inches (1 acre-inch is 1 inch of water applied to 1 acre); or (iii) gallons per minute (Gpm).

As an example, let's determine the water needs of a 100 acre vegetable farm. Suppose the farm has a peak use rate of 0.25 inches/day (this estimate can be obtained from a local agriculture expert). The farm would require 100 acres × 0.25 inches = 25 acre-inches/day. Most growers do not run irrigation systems 24 h a day, so suppose you calculate that you will run it for only 16 h a day. This would indicate a water use rate of 25 acre-inches ÷ 16 h = 1.56 acre-inches/h. As 1 acre-inch/h equals roughly 453 gallons/min, therefore, 1.56 acre-inches/h × 453 = 708 Gpm. No irrigation system is 100% efficient at delivering the water to the crop, therefore, this number of 708 must be adjusted to consider the efficiency rating of the system in question (Table 7.4).

Table 7.4. Water system efficiencies for several types of irrigation.

Irrigation system	Minimal efficiency (%)
Surface – basin	80
Surface – border strip	70
Surface – furrow	60
Sprinkler – portable	65
Sprinkler – traveling gun	60
Sprinkler – center pivot, linear move	75
Sprinkler – permanent	70
Trickle – point source emitters	75
Trickle – line source	70

If you were using trickle tape, for example, your source must be able to supply a minimum of 708/0.70 (70% minimum efficiency for line source trickle) = 1011 Gpm.

Water source

One of the first things to consider when selecting your water source from those available in your area, is whether or not you have the legal right to that water. Additionally, you must ensure that you have the right to draw the quantity needed, when you need it. If you must drill a well, make sure you have the right to do so.

Water sources include: (i) rivers and streams; (ii) lakes, ponds, and reservoirs; and (iii) wells. Rivers and streams often have variable flow rates depending on season and precipitation patterns. If natural rainfall is in short supply, river and stream flow is also likely to be reduced. If you have the right to the river or stream water and the flow is variable, you will probably require an on-farm storage facility. Lakes, ponds or reservoirs are good sources of irrigation water if you have the legal right to tap into them and the water flow into them is adequate. If none of these sources are applicable to your situation, you may need a well. Deep-drilled wells are usually most reliable for irrigation purposes. Again, you must make sure you have the right to drill a well.

Municipal water may be an expensive alternative. Additionally, consider that most sewer bills are calculated from water bills, so you'll be paying for sewage removal you are not using!

Once you establish your source of water, you must make sure you can get the water to your fields effectively. Consider distance, elevation, terrain, etc. The farther you are from the source, the more

pumping power you'll need. Pumps use electricity or diesel fuel, thus they are expensive to operate. Sometimes it's less expensive to select a different source (i.e. drill a well) than to pump water over a long distance.

Water quality

Water quality may not be consistent over the growing season, thus it is important to examine possible changes to water quality over time. Not only should natural changes be considered, but human-induced changes must also be examined. It is always a good idea to have your water tested often for natural and introduced contaminants. This includes testing for salts, industrial wastes, organic acids and bacteria from decaying plant or animal waste material.

Other contaminants you should consider include sand, silt, clay, and algae, particularly with trickle systems, as they are easily clogged. Filters are normally needed for trickle systems.

Irrigation systems

Selecting an irrigation system

Selecting an irrigation system should be a major part of the farm planning process. In planning the system, it is important to consider a number of issues.

Physical characteristics of the environment which may affect the operation and efficiency of an irrigation system are important. The soil is extremely important in selecting a system. Its texture, depth, and uniformity will affect infiltration capacity and rate, and drainage. Its initial salinity level sets the level for how much additional salt can be applied via irrigation water before substantial problems arise. Site characteristics such as topography indicate potential erosion issues. The crops which will be grown and the growing climate have a large impact on system selection. If crops will need frost protection utilizing the irrigation system, this must be incorporated into the decision. The source of the water must be considered. Is it high quality or does it have contamination issues with salts, other chemicals, or particulate matter? Is the water easy to get to and relatively close to the area of crop production? Is it a reliable source? If the system under consideration requires energy, is it readily available, reliable, and at a reasonable cost?

After determining if an irrigation system is feasible, economics of purchase, use, and maintenance must be studied. Most systems require a substantial initial investment of time and money. Once purchased and installed, time and money must be allocated for operation and maintenance.

If the site and economics are suitable, consideration must be given to legal and social issues. Permits must be obtained and the surrounding community must be favorable to the presence of the irrigation system. Is there potential for vandalism in the neighborhood? Labor with enough skill must be available for operation and maintenance of the system.

SURFACE IRRIGATION SYSTEMS

BASIN IRRIGATION Basin irrigation utilizes application of water to a completely level area surrounded by raised borders. The size of an area that can be irrigated with this system is limited by the amount of water available for one irrigation cycle. This type of system works well with large, level land parcels with uniform well-drained soil where adequate water is available. It is a fairly simple system with low labor requirements.

BORDER STRIP IRRIGATION Border strip irrigation incorporates land formed into strips that are level across the narrow dimension but slightly sloped in the long dimension and surrounded by a border. Water enters the field on the upper end of the border and flows down the sloped strips. While simple in conception, this is a very complicated system to manage.

While border length and slope are easily set, other factors entering the system such as moisture deficit at the time of water application, infiltration rate, and flow interference by the crop makes the system tricky to run.

FURROW IRRIGATION Furrow irrigation consists of furrows sloping down the length of a field to which water is applied. Water infiltrates vertically and horizontally. Many designs are possible with this method of irrigation. With good planning and management, this system can be quite effective. However, mismanagement can lead to undesirable results very quickly.

SPRINKLER IRRIGATION Sprinkler irrigation delivers water through a pressurized network to sprinklers, jets or nozzles which spray water into the air, creating

artificial rain. The basic components of this type of system are: (i) a water source; (ii) pump; (iii) piping network; (iv) control valves; and (v) sprinklers.

PORTABLE SPRINKLER IRRIGATION This type of system consists of a main supply line with lateral pipes to which the sprinklers are attached at regular intervals. The pipe is often lightweight aluminum 6–12 m (20–40 feet) in length with quick-connect joints for easy assembly and disassembly. Sprinklers are attached directly to the lateral pipe, or are on tall risers to raise them above the crop being irrigated, or on short risers to keep them below the crop canopy, as in orchards. Ordinarily one section of the field is irrigated at a time. The pipes must then be moved, and the next section irrigated. The initial cost of such a system is often low; however, labor costs for moving pipes can be high.

SIDE ROLL This is essentially a portable system mounted on wheels, with the lateral line serving as the axle. The wheels are high enough so that the lateral pipe clears the crop being irrigated. The wheels move via a gasoline-powered engine. The entire unit moves from one position to another for irrigating, rather than requiring constant disassembly and assembly.

TRAVELING GUN The travelling gun is a high-pressure sprinkler gun mounted on a trailer. Water is supplied via a flexible hose. The gun is moved to the far end of the field and water pressure operates a conveyor system which pulls the irrigating gun through the field by winding the supply hose onto a large wheel on the close side of the field.

CENTER PIVOT AND LINEAR MOVE This system consists of a single lateral on raised supports with wheels that rotates around a center point while irrigating the field. Water application rate must increase for the sprinkler units farthest away from the center of the field, since they move faster than the more centrally located sprinklers and must supply water to a larger area. Revolution rates of the unit can be from a half a day to several days. A problem with this system is that the corners of the field are not irrigated. A linear move system is similar to the center pivot, however, the entire lateral moves across the entire field rather than as a centrally pivoting lateral. Water is delivered to the moving lateral via a flexible source pipe. Both the central pivot and the linear move systems require high capital investment but have low labor requirements.

SOLID SET/PERMANENT A solid set system is essentially a hand-move lateral sprinkler system where enough laterals are placed in the field so that they do not have to be moved during the growing system. Water to each lateral is regulated by a series of valves. Solid set systems are removed from the field at the end of the growing season. Permanent systems consist of buried main and lateral lines, usually PVC pipe. They are not removed from the field at the end of each growing season and are most suited to perennial crops.

TRICKLE IRRIGATION Trickle systems deliver a low volume of water under low pressure from a main source line via lateral lines. Lateral lines utilize spaghetti lines with emitters, low-rise microsprinklers or trickle tape (Fig. 7.4). Generally the water is applied as close to the plants as possible over a long time at low rates. One of the biggest problems with trickle systems is clogged emitters caused by particulates or precipitates from salts in the water. Nearly all trickle systems require some sort of filtering device to remove particles from the irrigation water. Media filters are used to remove organic contaminants while chemical treatment might be required to control algal growth, adjust pH or to prevent precipitation of emitter clogging salts. Routine inspection is needed to look for leaks and to correct plugged emitters and lines.

Fig. 7.4. Trickle irrigation showing lay-flat supply hose connected to trickle tape via an adjustable valve for regulating water supply.

Water quality considerations

Water quality for use in irrigation is assessed by examining its salt content. In particular, the water should be analyzed for calcium, magnesium, sodium, bicarbonate, carbonate, sulfate, chloride, and boron. In addition, water may also be tested for chemical or biological contaminants. The two factors most often considered when evaluating irrigation water are: (i) total dissolved salts (TDS); and (ii) the sodium adsorption ratio (SAR). The TDS is a measure of the concentration of soluble salts in the water (ppm), often called salinity, and is reported as electrical conductivity (EC), measured as millimhos per centimeter (mmhos/cm), deci-Siemens per meter (dS/m) or micromhos per centimeter (μmhos/cm). Since TDS may be reported in different formats by different testing labs, it is useful to convert any report to a standard value for discussion using the following equations:

1 ppm = 1 mg/l

1 mmhos/cm = 1 dS/m = 1000 μmhos/cm = 0.1S/m

*TDS (*ppm) = *EC* (mmhos/cm or dS/m × 640

SAR is the ratio of sodium to calcium and magnesium in the water. The values of TDS and SAR that would indicate water unsuitable for irrigation vary with soil, irrigation frequency, and crop. Consult a local expert for interpretation of your specific values. However, in general most water with 200–800 ppm TDS and a SAR less than 3 can be used for irrigation. Levels of TDS above 2000 ppm or SAR above 3 may severely injure sensitive species. If sodium levels are provided, most species can tolerate sodium up to 70 ppm. Above that, injury occurs. Additionally, excessive sodium harms soil structure.

Sometimes calcium can be added to irrigation water as calcium chloride, calcium carbonate, or gypsum to lower SAR. The extent of SAR reduction depends on calcium solubility in the water which depends on source and the concentration of other ions in the water. Generally, calcium chloride is more effective in lowering SAR compared with gypsum and calcium carbonate, however, it is considerably more expensive.

Carbonate and bicarbonate ions form salts with calcium and magnesium in irrigation water, precipitating out and increasing the SAR and sodium hazard of the water. Generally the formation of these precipitates can be avoided or at least reduced by lowering the pH of the water with an acid.

The boron level of irrigation water should be monitored since some crops are extremely sensitive to boron. Levels which are normal for tolerant species can cause injury in sensitive species. If boron levels are high, crop selection may need to be adjusted to avoid injury.

Excessive iron from drilled wells can clog trickle emitters and may be toxic to some species. It can be removed with a reverse osmosis filter. Toxic chemicals in irrigation water need to be removed via a media filter. Chlorine and fluorine levels in municipal water are generally not high enough to cause plant injury. Allowing tap water to sit at room temperature overnight removes nearly all of the chlorine from water used for watering houseplants.

If the water source is municipal recycled water, it may contain excessive levels of chlorine which may be toxic to plants. Chlorine toxicity generally will only occur if high levels of chlorine are sprayed directly onto the foliage. This would only occur if the water used for irrigation has come directly from treatment at a recycling plant. Free chlorine is very unstable in water and will dissipate if water is stored for a brief period before use.

Chlorinating irrigation water

Biological contaminants such as algae, fungi, and bacteria must be addressed, particularly when present in water used for trickle irrigation. These contaminants are most likely to be found in surface irrigation water sources. Filters can remove much of the contamination; however, clogging of emitters is likely over time. Irrigation water can be treated with chlorine to sanitize the water and remove the source of the clogging. Chlorination is most often accomplished by injection of chlorine into the system as chlorine gas (dangerous and expensive), or a solution of sodium hypochlorite or calcium hypochlorite. When chlorine is added to water, hypochlorous acid and hypochlorite (both collectively known as 'free chlorine') form.

Liquid household bleach is sodium hypochlorite which normally has up to 15% available chlorine. Bleach is usually added to irrigation water via a fertilizer injector. The pH of the irrigation water is often raised by adding bleach, and the available chlorine is reduced. Acid may have to be injected to lower the pH in order for the bleach to be effective.

The injection rate for sodium hypochlorite can be determined via the formula:

$$IR = \frac{(0.006 \times Q \times C)}{S}$$

Where:
IR = the injection rate
Q = the flow rate (Gpm)
C = desired chlorine concentration (ppm)
S = strength of the sodium hypochlorite source (%)

Continuous chlorine injection is needed if the water is highly contaminated. The goal is to have 1–2 ppm chlorine at the end of the irrigation system. Testing for the chlorine level at the end of the system is important since organic matter or iron and manganese can remove chlorine from the water. A good swimming-pool test kit will do the job. Periodic injection of 10–20 ppm for 2 h may be enough if the contamination is less severe. Superchlorination injections of 500–1000 ppm can be used to reclaim drip systems clogged with algae or bacteria. Expert help for superchlorination is advised since damage to plants and irrigation equipment could occur.

Calcium hypochlorite contains 65–70% available chlorine with 12.8 lb (5.4 kg) of calcium hypochlorite dissolved in 100 gallons (378 l) of water forming a 1% chlorine solution. Thus any chlorine stock solution can be made using this information. Once the concentration is known, the injection rate can be calculated from the previous formula for bleach.

Generally, the chlorinated water should remain in contact with the biological target for at least 2 h. All systems should be flushed thoroughly before fertigation or before applying a pesticide through the irrigation system.

Physiology of Water Stress

Water stress, indicating an insufficiency of water, develops as a plant's water demands exceed the water supply. It may develop rather quickly or it may develop slowly over time (often called drought stress). It may also occur diurnally, which in reality is not true water stress, but rather the inability of a plant to transpire enough water to remain turgid.

On a normal day a plant absorbs an enormous amount of radiant energy from the sun. Some of this energy is utilized in photosynthesis while most of it is not used and must be dissipated. Some is re-radiated from the plant as heat, but most of it must be removed from the plant via transpiration.

As water evaporation cools a leaf, water leaving the leaf must be replenished with water from the soil via the xylem. If the water re-entering the leaf is less than that leaving it, cells begin to loose turgor and wilt. As leaf cells loose turgor, stomata begin to close, transpiration and photosynthesis rates decline, and leaf temperature increases. Without transpiration, the temperature of the leaf can reach high enough levels to cause damage.

Turgor can be maintained by replenishing water lost from the leaf through transpiration or by adjusting the osmotic potential of leaf cells by accumulating solutes such that water is held more tightly by these cells. This process is called osmotic adjustment (OA) and is one of the first physiological responses to water stress. Cell walls can harden to maintain turgor, however, once hardened, cell growth by expansion cannot occur.

Besides cell turgor, ABA is a potent transducer of the water stress signal. As water becomes more limiting, root tissue in direct contact with drying and hardening soil produces ABA which is then transported in the xylem to the shoot. Some roots may remain moist while others dry out producing ABA and exporting it to the shoot. Thus some of the first shoot responses to water stress are induced long before the leaves sense a reduction in water supply. The stress signal from the root induces closing of stomata and an overall reduction in growth. This is a classic example of hormonal signal transduction in plants. As leaves desiccate in response to the stress, ABA is produced, amplifying the stress responses by the plant.

On the whole plant level, transpiration rate is regulated by total crop leaf area. One of the plant level responses to water stress is desiccation and abscission of older leaves, thereby reducing total leaf area and therefore the amount of water required for transpiration. A common measure often used in discussing water stress on a whole plant or crop level is the leaf area index (LAI). LAI is the total live leaf area per unit land, expressed as square meter per square meter (m^2/m^2). Transpiration increases with LAI. As a crop develops and matures, leaves abscise and the LAI as well as transpiration on a land unit basis also decreases.

Measuring plant water stress and water status

Leaf temperature is a decent quick estimate of plant water stress. The crop water stress index (CWSI) is a measure of the amount of water stress a plant canopy is under. The canopy can be a single plant or an entire field. The index is developed for irrigation scheduling using infrared temperatures of plant leaves. As transpiration decreases in response to water stress, leaf temperature relative to air temperature increases.

Leaf water potential (LWP) can be directly measured as an indication of water status. When a leaf is cut from a plant, sap under tension is pulled back into the xylem with a force equal to but opposite in sign to the LWP. The cut leaf is placed in a pressure chamber with the cut end exposed. As pressure is applied to the chamber, xylem sap is forced out of the cut petiole. When the sap appears at the petiole end, a pressure reading is taken and the LWP is estimated. LWP of live transpiring leaves is generally in the range from -0.3 MPa to -2.5 MPa.

Another piece of equipment used to estimate leaf water status is the thermocouple psychrometer. A leaf is sealed inside a small chamber with an attached thermocouple. After the sample has equilibrated in the chamber, a current is applied to the thermocouple causing it to cool. As it cools, water will eventually condense on it. More water in the leaf sample results in more water condensing on the cooling thermocouple. The current is turned off and the water evaporates which generates a signal in the thermocouple. A calibration curve is developed using various salt solutions such that the LWP of the leaf sample can be estimated.

The turgor pressure of a single cell can be measured directly with a small microcapillary tube filled with oil. The oil in the capillary moves back in proportion to the cell's turgor pressure. An estimate of the pressure on the capillary can be made by applying a balancing pressure to the capillary.

Relative water content (RWC) is often used as a measure of plant water status and is often preferred over LWP since RWC accounts for any effect OA might have on leaf water content. Two plants can have the same LWP but different RWC due to the presence of OA in one of the plants. OA involves the production of solutes by the plant which increases the osmotic potential in order to maintain higher LWP.

Osmotic potential can be measured by thermocouple psychrometry of tissue which has been frozen then thawed which releases all the solutes in the cell. Killed tissue cell sap can also be measured for osmotic potential using a micro-osmometer. Turgor can then be estimated as the difference between LWP and osmotic potential as long as both the LWP and the osmotic potential are measured using the same technique. This removes error that might be introduced by using two different techniques.

In order to estimate the amount of OA a plant undergoes during water stress, one set of plants must be carefully stressed and water stress estimated via RWC and osmotic potential measured using one of the previously described methods. The other set of plants are kept fully hydrated. OA is estimated as the difference in osmotic potential between stressed and non-stressed plants and often ranges from 0 to 1.5 MPa. Another method involves applying a predetermined amount of stress to a plant followed by overnight rehydration. Osmotic potential is then measured after rehydration and compared with a plant that was not stressed and kept fully hydrated. The OA is the difference between the osmotic potential of the two plants.

Measuring transpiration and stomatal conductance

Numerous methods have been devised for estimating transpiration rates and stomatal conductance. Gravimetric measurements simply rely on measuring how much water a plant uses in a closed system over time and expressing it as mass of water used per unit of leaf area per unit of time. Porometers can be used to estimate the transpiration of single leaves. The most common device of this type is the diffusive resistance porometer. With plants that have leaves which are difficult to reach, such as tall trees, the measurement of a pulse of heat up the tree through the xylem is measured to estimate transpiration rate.

Plant responses to water stress

Plant responses to water stress are complex and involve either adaptive changes in metabolism and overall plant structure or deleterious effects such as reduced photosynthesis and yield or leaf abscission. Coping strategies are often a mix of stress tolerance and avoidance mechanisms.

At the molecular level, there appears to be two signaling pathways in the perception and transduction of water stress, one involving ABA and the other not involving ABA. The ABA-dependent pathway involves the production of proteins in response to the water deficit which regulate genes responding to the stress. ABA may also interact with these genes without protein synthesis. The gene products can be functional or regulatory. Even though there are hundreds of drought responsive genes that have been identified, how they work to protect a plant from the deleterious effects of water stress are yet not clear.

While the immediate responses to water stress occur at the molecular level, the repercussions of such genetic regulation have profound effects on plant growth and development, and ultimately, yield. Probably the most sensitive plant response to a water deficit is reduced cell expansion. If cell expansion is reduced, growth is reduced and the water requirement is lowered. However, concomitant with reduced growth and water requirements is a reduction in yield. If the reduced growth isn't enough to maintain turgor and prevent wilting, stomata will close, reducing water loss and carbon uptake, further reducing yield.

A reduction in cell expansion also impacts meristematic tissues further reducing yield. Reduced cell expansion in meristems may lead to reduced branching and smaller reproductive organs. These effects are irreversible since once a reproductive organ has passed a certain point in its ontogeny, size is set and cannot be altered.

Flowering can be delayed or advanced depending on species. Rice flowering can be delayed by as much as 50 days by a pre-flowering water stress and this delay may be due to ABA. Water stress delays ear development but not silk development in many cultivars of corn (maize). As such, the time between silking and anthesis is greatly lengthened, with a likely reduction in yield due to inefficient pollination. A major feature of drought resistant corn is a short anthesis-to-silking interval. Pollen development is affected by drought stress with sterility often occurring under stress conditions. Another possible cause of reduced yield associated with water stress is reduced sugar production in the leaves and also translocation to developing ovaries, grain, or fruit. Root growth may or may not be enhanced by water stress. Under stress, roots of some species grow deeper into the soil.

Drought resistance

Drought resistance is often discussed as two components: (i) dehydration avoidance; and (ii) dehydration tolerance. Dehydration avoidance is the capacity of a plant to avoid dehydration at the cellular and tissue level under conditions of water stress. Dehydration tolerance is the capacity of a plant to function relatively normally under conditions of water stress. Rather than categorize plant responses as those that adopt avoidance or tolerance, we will address the plant responses that are most associated with water stress.

Roots

Deeper root systems and greater root length are both features which may allow certain species to avoid dehydration. Water-stress induced ABA maintains root elongation under drought conditions by suppressing ethylene production in the roots.

Leaves

One of the first major plant responses to water deficit is closing of stomata, which reduces the rate of water loss and carbon uptake by the leaves. Stomata close in response to ABA produced in the roots and transported to the leaves under increasing water stress. As the water stress intensifies over time, stomatal closure occurs for longer and longer periods during the day, usually starting mid-morning. With stomata closed for a longer and longer period each day, carbon fixation is reduced as well. This can ultimately lead to reduced growth and yield. In addition to stomatal closure and reduced carbon fixation due to low carbon dioxide availability, long-term carbon fixation also declines due to a slow reduction in the activity of enzymes of the Calvin cycle.

Species that are resistant to water stress show remarkable resilience in photosynthesis following rehydration after stress. In addition, photosynthesis is often less inhibited by very high temperatures (38–40°C) in dehydrated plants compared with well-watered plants, especially in resistant species.

While most of the water lost by plants is through the stomata, some transpiration does occur directly through the leaf surface. Thus, some species avoid dehydration by having very thick cuticles on their leaves. In addition, the shape of the wax crystals which are part of the cuticle can reflect solar radiation,

reducing the amount of energy that must be dissipated by transpiration. On the same lines, pubescent plants often have lower leaf temperatures and a lower water requirement than glabrous plants. This is due to several factors. The hairs create a modified boundary layer reducing water loss from leaves. In addition, the hairs reflect radiation, resulting in cooler leaves and reduced transpirational needs.

General plant stature/development

Dehydration avoidance can be accomplished by small plant size. Small plants tend to have small leaf areas and they tend to use less water. Many species that inhabit xeric landscapes are small with small leaves.

Even though water stress can lead to reproductive failure, some species avoid seasonal water stress by flowering and fruiting before the stress occurs. Other species can resist water stress during reproduction by accumulating reserves in stems and roots as soon as any stress is detected by the plant. The reserves are remobilized during reproduction.

Osmotic adjustment (OA)

A major physiological mechanism of dehydration avoidance is OA. OA results from a net increase (not just an increased concentration of) in cellular solutes, particularly potassium, sugars, and amino acids (particularly proline). OA is different in different species and develops over a long time (2 weeks). By lowering the osmotic potential of the cytoplasm, water is held more tightly by the cell, reducing or avoiding dehydration. In addition, the solutes protect proteins, enzymes, and membranes against desiccation injury. Upon rehydration after the stress, some of the solutes, particularly sugars, serve as an energy source for the recovering plant.

Oxidative damage

Some of the damage caused by water stress is from oxidative damage. Reactive oxygen species (ROS) are produced from energy not dissipated in the leaf by photosynthesis or cooling via transpiration. One of the protective measures observed in plants under water stress is a large rise in both enzymatic (superoxide dismutase, ascorbate peroxidise, and glutathione reductase) and non-enzymatic (α-tocopherol or diterpenes) antioxidants in the leaves.

Drainage – getting rid of too much water

Sometimes too much water is in the soil. Excessive water can induce as much stress to plants as too little. In this section we will examine the most widely used methods of draining excess water from agricultural land. Too much water might be the result of excessive irrigation or inadequate drainage or a combination of the two. Excessive irrigation is easily controlled with good management. Poor drainage must also be addressed (Ritzema, 1994).

Generally, if the problem is surface ponding of excess water, shallow, sloping, open drains are dug to remove the excess water. Waterlogged soil often requires a more extensive approach, often involving installing subsurface pipes to drain the water away. In either case, there are many benefits to removing excess water from production fields including: (i) a greater choice in what crops will grow; (ii) increased production; (iii) roots can grow more deeply, reaching nutrients farther down in the soil; (iv) better growth results in more efficient use of nutrients; (v) the soil structure will be enhanced over time; (vi) there are more windows of opportunity for field work; and (vii) the soil warms more quickly. Even if you have the idea that "it's not that waterlogged" or "plants are tough, they can take it", you should consider these benefits to removing excess water.

Components of a drainage system

There are three main components to any drainage system: (i) the field drainage system; (ii) the main drainage system; and (iii) the discharge outlet.

The field drainage system gathers excess water from the land using field drains and field modifications such as field sloping to promote the flow of water off the land and into the main drain. It can be surface or subsurface, and the subsurface drain can be open or a system of pipes.

The oldest and simplest form of surface field drainage is bedding. Raise beds are constructed in the field such that excess water collects in the divots between the beds and flows to a main drain. The land must be sloped to allow water movement. Beds can be formed and shaped in various configurations using manual labor, animals or mechanical

equipment (Fig. 7.5). If bedding is insufficient, land grading, and planning might be needed to achieve the desired slope for water removal. Land grading is a process in which the surface is formed to specific grades so that each field slopes towards a field drain. Compared with bedding, grading requires fewer field drains. Envision a field in which you form 20 beds, each with a field drain running to a collecting drain at the end of the field. The same field might be graded such that you only need two field drains, one on each side of the field, to carry the water away. Land planning smooths the field to remove minor imperfections in the surface topography which removes dips and depressions where water might collect.

Subsurface drains are either open systems or a system of pipes. Open drains have the advantage that they can collect excess surface water and thus also serve as surface drains. Their disadvantage is that they require constant maintenance and they use a large amount of land area and make traversing a field with equipment difficult. Pipes have the disadvantage of being expensive and they may require extensive excavation maintenance if a pipe should break or become clogged. The advantage is that once installed, maintenance is low and not much land area is removed from production.

The main drainage system receives water from the field drains and moves it to the outlet. It also collects and moves surface runoff and excess groundwater. The main drain is often a canal or series of canals. They can be open or a series of pipes.

The outlet is where the collected water is discharged into a river, lake or the sea.

Fig. 7.5. A mechanical bed maker for pulling behind a tractor. The attachment makes beds 1 m wide and 20 cm high providing an elevated rooting environment for crops. Raised beds are often a sufficient remedy for minor drainage problems.

Drainage requirements

When designing a drainage system, it is imperative to know the requirements for drainage of each field under consideration. The primary consideration must be determining the volume of water that must be removed from the field to enhance production. This is often not easy to do. Seasonal and year-to-year differences must be considered. The depth and extent of the water table must be evaluated. The area in the soil where water pressure is equal to atmospheric pressure is the upper level of the water table and soil pores are saturated with liquid water. Just above the water table is a region of partially liquid saturated soil, from which plants retrieve most of their water. If excessive drainage is employed, this partially saturated zone will be too low and crop growth will suffer. If drainage is insufficient, this layer will be too high and plant roots will be waterlogged. Further complicating the issue is the fact that the water table varies over time. Groundwater may be saline depending on field attributes. This must be considered in drainage plans, since salty groundwater often results from excessive salts in the soil which must be routinely leached and drained away, especially in irrigated arid climates.

A soil's hydraulic conductivity, a measure of how well a soil transmits water, is important. If a soil has low hydraulic conductivity, drainage will be more difficult.

A field's topography is important since most of the water is drained away using gravity. In addition, knowledge of any impermeable layers in the soil must be taken into account.

Detailed records and maps should be developed during construction so that future occupants of the land will know of any underground components. Notations of alterations and maintenance should also be kept.

Operation and maintenance

After installation, a drainage system must be properly operated and maintained. Unless pumps are involved, much of the operation occurs automatically. Frequent observations of the system while it

is operating must be made. The system should also be inspected while idle to catch and address any problems as soon as possible.

Physiological problems caused by too much water

Even though greater than 90% of a plant's makeup is water and water is absolutely essential for survival, even a small excess of water, especially in the root zone, can be lethal to plants. While there are a number of species that are quite tolerant of waterlogged soils, most crop plants do not tolerate excessive water in their root zone for any extended period. This is because water is very impermeable to gas exchange. Plant roots need oxygen to survive and function, and waterlogged soil prevents roots from receiving adequate oxygen for metabolism. It has been estimated that about 10% of farmland worldwide suffers from frequent waterlogging, reducing crop productivity by 20%.

The major consequence of waterlogging is inadequate oxygen supply to root tissue. Diffusion of oxygen through water is only about 3% that of diffusion through air, thus water-filled soil pore spaces prevent sufficient oxygen transport to root tissues. In addition, the waterlogging prevents the diffusion of ethylene and carbon dioxide away from the roots. Carbon dioxide in the soil solution can damage roots of some species (*Glycine max*) but not others (*Oryza sativa*). Waterlogging can also reduce nitrogen and iron availability in the soil and increase the levels of highly soluble manganese (Mn^{2+}) and iron (Fe^{2+}) ions which may be toxic to membranes and interfere with the activity of some enzymes. Anaerobic bacteria may convert SO_4^{2-} to H_2S, which is toxic to respiratory enzymes and non-respiratory oxidases.

Oxygen deficiency

Death of root tissue from oxygen deficiency is caused by insufficient ATP production at low oxygen levels followed by poisoning by products from anaerobic respiration. Soil water in equilibrium with air contains approximately 0.25 mol/m^3 of oxygen at 25°C, thus initially there is some oxygen in water close to the surface of waterlogged soils. Oxygen diffuses very slowly in water, thus the little bit of oxygen initially in waterlogged soil is quickly utilized.

ATP insufficiency

Aerobic roots generate sufficient ATP through aerobic respiration (38 molecules of ATP per molecule of glucose). When roots become anaerobic, ATP is generated by glycolysis (two molecules of ATP per molecule of glucose). Glycolysis also feeds pyruvic acid into fermentation producing ethanol, a potent toxin. Unless metabolic processes are limited to only essential life-supporting processes, the ATP formed through glycolysis is insufficient to maintain cell function. Most plants cannot regulate which cellular processes receive ATP and which do not, thus the lack of sufficient ATP production from glycolysis under anaerobic conditions is lethal. Under these conditions, the cytoplasm and vacuole become very acidic with negative impacts on membrane integrity. Once membrane integrity is compromised, the damage is irreversible.

The ATP produced from glycolysis relies on a supply of glucose. Quickly after anaerobic conditions commence, starch breakdown and phloem unloading cease, limiting the supply of glucose, further limiting the supply of ATP. If cells are fed glucose under anaerobic laboratory conditions, they still die, indicating that lack of a substrate for ATP production is not the sole source of death in these cells. Lack of oxygen itself for other metabolic processes contributes to cell death.

Self-poisoning

Roots under anaerobic conditions may die from self-poisoning caused by products of anaerobic metabolism. The number one toxin produced under anaerobic conditions is H^+ which causes acidification of the cytoplasm and vacuole which leads to compromised membrane integrity.

Acetaldehyde is another potential toxin. Acetaldahyde is produced from pyruvic acid by pyruvate decarboxylase. Acetaldehyde is then converted to ethanol by alcohol dehydrogenase. The rate of ethanol production from acetaldehyde usually exceeds the conversion of pyruvate to acetaldehyde. When tissue is returned to aerobic conditions, this regulation is sometimes lost and a toxic level of acetaldehyde may develop.

Nitric oxide, a free radical gas, formed by nitrate reductase under anaerobic conditions may be toxic enough to kill root tips. Some scientists suggest that death of root tips by nitric oxide might be a survival mechanism under anaerobic conditions.

Root tips may die, but the rest of the root can survive if the anaerobiosis is not too long. Once the anaerobiosis is gone, dormant buds on surviving roots can begin to grow, replacing damaged tips.

Surviving anaerobiosis

The level of oxygen in the soil and soil water slowly declines as a soil becomes waterlogged subjecting roots to a period of hypoxia which may induce biochemical or anatomical changes which support survival of anaerobic conditions.

BIOCHEMICAL ADAPTATION If plants are exposed to slight soil oxygen stress for 6 h before aerobic conditions, survival is increased from 8 h to 72 h in some species. The mechanism for sensing the slight stress is unknown. The slight stress induces the production of anaerobic proteins, enzymes involved in: (i) energy metabolism; (ii) pH regulation; (iii) aerenchyma formation; (iv) ROS scavenger production for when roots return to aerobic conditions; (v) signalling; and (vi) yet others which have unidentified functions. If the synthesis of these proteins is inhibited, the acclimation reaction is not observed. Concomitant with production of these enzymes is a coordinated reduction in the demand for oxygen, substrates, and ATP.

ANATOMICAL ADAPTATION – AERENCHYMA FORMATION Species that grow well in wet soil often have very large intercellular gas-filled spaces all the way from the shoot to the root tip. This tissue is called aerenchyma tissue. The air spaces are created by differential division and expansion of adjacent cells or by death of certain cells. Enough oxygen can diffuse through aerenchyma tissue to supply the roots up to 30 cm from the shoot with sufficient oxygen for metabolism.

Most species do not possess aerenchyma tissue. There are a few species (e.g. *Z. mays*) that can produce aerenchyma in roots during the early stages of reduced oxygen supply during waterlogging. The aerenchyma formed is a result of cell death in the root cortex that is stimulated by increased ethylene concentration. The increased ethylene concentration is due to increased production and reduced ethylene catabolism. Death of specific cells begins in about 6 h of elevated ethylene and is complete within 2 or 3 days. Cell death is an ordered sequence of events which is not well understood.

SHOOT TOLERANCE OF LOW SOIL OXYGEN LEVELS Shoots and roots are intimately linked, so it is no wonder that low oxygen levels in the roots eventually affects physiology of the shoots. Since nitrogen uptake and metabolism in the root is curtailed with waterlogging, young leaves begin to utilize nitrogen remobilized from older leaves. The older leaves senesce prematurely. Waterlogged plants also tend to wilt (not epinasty) under bright light due to a reduced capacity for water uptake by roots in a low oxygen environment.

Rapid signaling from the roots to the shoots during waterlogging decreases stomatal opening and leaf expansion. In some species like tomato (*Lycopersicon esculentum*) a marked epinasty of leaves occurs. Epinasty is the downward 'wilting' appearance of leaves under excess water conditions caused by increased cell division on the adaxial (upper) side of the leaf petiole. This reduces the angle of light incidence on the leaf blade, reducing water loss.

How are leaves signaled to become epinastic? Roots under anaerobic conditions export the ethylene precursor 1-aminocyclopropane-1 carboxylic acid (ACC) to the shoots via the xylem. ACC is exported to the shoots due to increased ACC synthesis and reduced oxidation of ACC to ethylene in the roots as a result of low oxygen in the roots. ACC builds up in the root tissue and enters the transpiration stream. Finally, leaf petioles have an enhanced ability to oxidize ACC within 6 h of waterlogging, caused by enhanced ACC oxidase synthesis in the petiole.

In waterlogged conditions, shoot bases of some species undergo changes that may enhance survival of roots under anaerobic conditions. These changes include development of aerenchyma tissue in lower shoots or hypertrophic lenticel development in water-covered shoot bases. Lenticels are groups of specialized cells on stem, root, or fruit tissues, much like stomata on leaves, which enhance gas exchange. Hypertrophic lenticels are enlarged lenticels. Another response often involves the production of replacement roots near the surface of the soil. Replacement roots near the soil surface may occur through the stimulation of growth in preformed root initials or the initiation and subsequent growth of root initials at the stem base. Both processes are induced by ethylene. Another root growth alteration to help survival in anaerobic conditions is the upward growth of lateral roots towards the soil surface.

Using Water to Regulate Temperature

Evaporating water uses energy. This energy is removed as heat from the object water is evaporating from. This fact is utilized in several areas of horticulture including: (i) postharvest produce cooling; (ii) cooling of greenhouses; (iii) cooling of growing crops in the field during high temperatures; and (iv) reducing heat unit accumulation in orchard trees to delay bloom.

Cooling with evaporation requires an understanding of wet bulb and dry bulb temperatures and their impact on evaporation. The dry bulb temperature is the temperature we all know as the air temperature. It is readily available from weather reports or from a thermometer. The wet bulb temperature is the lowest temperature the air could be cooled to via evaporation. When your skin is wet and the wind is blowing, the temperature you perceive cooling is the wet bulb temperature. The wet bulb temperature is a measure of the amount of water in the air. Wet bulb temperatures are available from the local weather station or measured directly with an aspirated or sling psychrometer.

A psychrometer consists of two thermometers. One is called the dry bulb. The other is called the wet bulb and has a cotton wick surrounding its bulb and the wick is immersed in water. Air is forced over both bulbs either by a fan (aspirated psychrometer) or by twirling the unit (sling psychrometer) to create a stream of air. The air passing over the wet bulb wick evaporates water until the wet bulb temperature is reached. The other bulb supplies the dry bulb temperature.

If a psychrometer is not available, the wet bulb temperature can be calculated from the RH, which is easily obtained from a weather station. (RH can be measured with an electronic humidity meter, but these often need to be recalibrated quite often. A psychrometer is the better choice.)

Knowing the wet bulb temperature provides an estimate of the maximum cooling potential of evaporation.

Cooling produce

Evaporative cooling can be used to cool harvested produce. It can be used for the initial removal of field heat from the commodity and it can be used to cool produce during storage. The cooling process involves misting the produce, usually in the presence of air with 65% RH or less. The maximum temperature reduction possible through evaporative cooling is 6–8°C, and the amount of cooling actually achieved can be quite variable. A general rule of thumb is that you can cool to about 1 or 2°C above the wet bulb temperature of the air.

To minimize the amount of field heat that must be removed, it is best to harvest early in the day when field heat is minimal. Keep the produce as cool as possible until it can be brought to storage. Produce can be pre-cooled prior to storage by misting it and allowing the water to evaporate. Even though this may only reduce the produce temperature by a few degrees, it is an inexpensive method for reducing the cooling load of the permanent storage cooler. It certainly is less expensive than other pre-cooling methods such as forced air, hydrocooling, or vacuum cooling.

Cooling greenhouses

Most greenhouses reach excessive temperatures during the summer growing season and are unfit for crop production unless cooled. Evaporative cooling is the most widely used method of greenhouse cooling (Bucklin *et al*., 2011). Air conditioning can be used, but installation and operation costs are prohibitive.

To obtain a measure of potential cooling with evaporation, obtain the wet bulb temperature in the early afternoon. This is when maximum cooling will be required. With a well-managed and maintained system, the greenhouse temperature can be cooled to within 1 or 2°C of the wet bulb temperature.

Evaporative cooling of greenhouses is accomplished by evaporating water into an airstream. This is most often accomplished using what is called a fan and pad system. High pressure fogging systems can also be used to evaporatively cool a greenhouse. They provide more uniform temperatures and humidity in the house, but are substantially more expensive.

The fan and pad system consists of an exhaust fan located at one end of the greenhouse which draws outside air through a vent at the other end of the greenhouse. As the air enters the greenhouse through the vent it passes through a porous pad, usually made of cellulose, through which water is trickled with a circulating pump. It is important to have a tightly sealed greenhouse so that air enters only through the pad to maximize evaporation. Water evaporates and cools the air

entering the greenhouse. Each gallon of water evaporating removes 8100 BTUs (British thermal units) of heat from the air entering the greenhouse. Air is coolest immediately after passing through the pad and entering the greenhouse and warms slightly as it approaches the fan. The temperature gradient should be as small as possible. Air may warm by as much as 1°C every 6 m it travels. Additionally, the cooled air tends to travel in an angle up and away from the plants it was intended to cool. In a cross-greenhouse flow configuration, this is usually not a problem, as gutters connecting roof sections of large greenhouses provide baffles to deflect cool air downwards. In smaller greenhouses, the distance from side to side is short, thus the air divergence is not of great concern. In lengthwise-flow configurations, baffles should be created every 10 m and extend from the roof of the greenhouse down to just above crop level.

If the efficiency of the cooling system is known, the temperature of the air exiting the cooling pad can be estimated as follows:

$$T_{cool} = T_{out} - (\text{Percentage efficiency})(T_{out} - T_{wb})$$

Where:
T_{cool} = the temperature of cooled air
T_{out} = the temperature of outside air
T_{wb} = the wet bulb temperature of outside air

A well-designed system can have an efficiency of up to 85%. To illustrate how effective pad cooling systems can be, a system with 85% efficiency can take outside air at 32.2°C and 50% RH down to 24.7°C. That's a 7.5°C decrease in temperature.

Regulating bloom in fruit crops

Many deciduous species require exposure to moderately low temperatures (0–5°C) for a species-specific duration (chill units) in order to complete endodormancy. After fulfillment of this chilling requirement, buds will grow and develop upon exposure to warmer temperatures. Evaporational cooling with water can be used to increase chill units accumulated or reduce heat unit accumulation. Increasing chill units promotes bloom by terminating endodormancy, while reducing heat unit accumulation delays bloom to avoid frost or freeze injury (Alfaro *et al*., 1974; Anderson *et al*., 1975; Bauer *et al*., 1976; Buchanan *et al*., 1977).

Terminating rest in fruit crops

Deciduous fruit crops are often grown in climates marginal for chilling. Instead of switching to other crops, attempts have been made to artificially provide chilling. One method is to reduce solar energy absorption by buds with reflective materials such as white latex paint or kaolinite clay. This approach has met with very limited success (Glenn *et al*., 2002). Another method uses evaporational cooling with water to reduce bud temperature, thereby increasing chill unit accumulation to break endodormancy (Gilreath and Buchanan, 1979; Hewett and Young, 1980). Overhead irrigation or misting can cool dormant buds by 4–6°C during chilling accumulation. Bud break following overhead irrigation to enhance chilling is often accelerated by a week or so compared with non-irrigated controls. This may be a response to increased chilling or may be a response to leaching of water-soluble plant growth regulating substances from the buds.

Drawbacks to this method are: (i) the expense associated with irrigation; and (ii) the potential for increasing plant susceptibility to frost and freeze injury and disease.

Delaying bloom in fruit crops

Evaporational cooling has been considered as a means for reducing heat unit accumulation early in the spring thereby delaying bloom in some fruit crops (Chesness *et al*., 1979). Temperatures of buds can be reduced to nearly the wet bulb temperature and bloom has been delayed by as much as 10–18 days depending on species.

As in irrigating for enhanced chill unit accumulation, potential problems with irrigating to delay bloom include: (i) the expense of irrigating; (ii) orchard waterlogging; and (iii) increased susceptibility of sprinkled buds to freeze damage and disease.

Crop cooling

Some fruit, particularly apples (*Malus domestica*) and brambles (*Rubus* spp.), may be subject to sunburn. This occurs when the fruit surface temperature is too high for an extended period. Even 1 day of excessive fruit temperatures can cause damage. Sunburn appears as a discoloration of the fruit skin. In brambles, individually burnt drupelets appear white. One method for reducing

fruit surface temperature is by overhead irrigation (Parchomchuk and Meheriuk, 1996).

Fruit can be cooled by convection, which is cooling of the air by evaporation of water into the air (Unrath and Sneed, 1980). The cooled air then cools the fruit. Fogging systems cool by convection, but they must be run continuously to be effective. Fruit can also be cooled directly by applying cold water to the trees in fruit. The cold water absorbs heat from the leaves and the fruit, thereby cooling them. Overhead irrigation provides this type of cooling. It does, however, require a tremendous amount of water which may lead to other problems (waterlogging, water wastage, nutrients leaching from the soil). The most effective method of cooling fruit is using evaporative cooling. This method of cooling removes heat from tissues by evaporating water off their surfaces.

An effective cooling system for fruit in the field should strive for a maximum amount of evaporative cooling, since it is most effective. The effectiveness of the system depends on: (i) RH; (ii) wind speed; (iii) water application rate; (iv) temperature; and (v) grower ability. Growers must be able to ensure that the system can be run for the duration of the potential sunburning season. Even 1 day missed could effectively wipe out the entire season's efforts for reducing sunburn.

Fruit injury can occur if the fruit temperature is at or above 30°C. Fruit temperature should be monitored just below the skin on the sunny side of the fruit. Fruit in full sun can reach temperatures as high as 50°C even when the air temperature is much lower. Careful monitoring of fruit temperature is critical. The system should be turned on when the fruit temperature reaches 2°C below the critical temperature. Thus in general, the system should be turned on when the fruit temperature is 28°C. The system should be turned off when the fruit temperature falls below 28°C.

The idea of fruit cooling is to keep the fruit from reaching the critical temperature, not cooling the fruit down once it reaches that temperature. The irrigation system must be able to supply enough water to keep the fruit below the critical temperature. The on-off cycle timing for the system should be such that maximum evaporative cooling occurs with a minimum amount of water application. Application of water in pulses to keep the fruit surface wet so that water is always evaporating from the fruit surface. Excessive water application does not enhance cooling and is wasteful of water and energy.

Red fruit color in apple (*M. domestica*) can be improved with fruit cooling in some growing regions. Fruit color is enhanced in 70–80% full sun with day/night temperatures both less than 27/10°C, respectively. Generally, cooling for this purpose begins 4 weeks before harvest. Cooling should commence 30 min before sunset and last until 60 min after sunset. In addition to improved color quality, crop cooling increases soluble solids, fruit firmness, and average fruit weight. It has also been shown to reduce corking and bitter pit in apple.

Crop cooling may leave chemical residues on the fruit after evaporation. Most often, the residue of concern is calcium carbonate. Analysis of the water used for cooling will provide clues as to whether or not precipitates left after cooling might be a problem. Some of the following indicate a potential problem: (i) a pH of 7.8; (ii) EC above 3 mmhos/cm; (iii) calcium or magnesium concentrations of 50 mg/l; and (iv) carbonate or bicarbonate levels of 100 mg/l.

Water requirements for crop cooling must be considered when calculating total crop water requirements. Water used for crop cooling does not supply appreciable water as irrigation. Thus, water used in cooling must be subtracted from the total water available, thereby reducing the water available for irrigation. Since most growers have a finite water supply, drip irrigation is a good way to save irrigation water, making it available for cooling.

Frost protection using water

Water in plant cells is not pure. It contains dissolved sugars, salts, and proteins. Thus the freezing point of plant cells is usually one to several degrees below 0°C. Most plant tissues are not injured at 0°C, thus keeping a plant at a temperature very close to 0°C can prevent freezing injury.

Overhead irrigation is used to prevent frost injury to fruit crops in many fruit growing regions of the world. Several important properties of water make this possible. One is that approximately 1 calorie of heat must be removed per gram of water for each 1°C reduction in temperature. In other words, water gives of a little energy as it cools. The second property which is much more significant in frost protection strategies is that 80 calories of heat are released for each gram of water freezing when water freezes at 0°C.

A third factor which is often overlooked in discussions on frost protection with irrigation is the

fact that when both solid and liquid water comingle, the temperature of the mixture will neither go above nor below 0°C until the phase change is complete. That is, the temperature stays at 0°C until all the water freezes or all the ice melts. The basic idea of frost protection with water is to maintain a thin film of water on the plant, even if the film is over a layer of ice. As long as the film of liquid water is present, the temperature of the tissue being protected will not fall below 0°C. Contrary to popular belief, the freezing water does not warm the plant or the air around it.

The plant must be able to support the weight of ice formed during protection. With low-growing crops such as strawberry (*Fragaria* × *ananassa*) this is generally not a problem. With tree fruit such as peach (*Prunus persica*) or apple (*M. domestica*), this can be a significant concern.

The protocol for frost protection with irrigation must be followed precisely for success. Irrigation must be turned on early enough in the evening to compensate for the evaporational cooling of the air that is likely to occur when the system is first turned on. Adequate volume of water application must be maintained during the frost event to ensure a thin layer of liquid water on the crop as sprinkler heads revolve during application. If too little water is applied, the phase change to ice may be complete before the next quantity of liquid water is applied, and the temperature of the plant may drop below freezing causing injury. Finally, a liquid layer must be maintained until all of the ice has melted following sunrise. If the irrigation is turned off too soon, the temperature of plant tissue may fall below freezing due to evaporational cooling or cooling caused by ice sublimation.

Undertree misting or irrigation can be used for frost protection but it does not afford the same level of protection as overcrop irrigation and it works via a different mechanism. Undertree systems operate by applying a film of water near the surface of the soil via short misting or irrigating risers. The water freezes releasing heat energy which is then transferred to the crop above. In addition, the layer of ice that forms on the soil surface reduces radiational cooling, keeping the orchard warmer than non-irrigated orchards. Tissues are not encased in ice as with overhead systems, and the system does not operate continuously. Undertree systems provide only about 1–3°C protection and are effective only if the wet bulb temperature is near 0°C. Colder dew points lead to excessive evaporational cooling which could totally negate any protection afforded by the freezing water.

In general, overtree irrigation may provide up to 6.7°C protection while undertree systems may provide up to 2.8°C protection. The advantage of undertree systems is that water application can be pulsed rather than applied continuously, thereby reducing water use. In addition, undertree systems can also take advantage of smaller nozzles which also reduces water use. While smaller nozzles can be used with overhead systems, their use is tricky as they may not provide sufficient volume and coverage.

Mass Flow and Movement of Assimilates in Plants

The movement of assimilates in the phloem in plants is investigated in this section on water since the movement of phloem solutes depends on water and is intimately connected to water in the xylem. Plants move assimilates (primarily sugars) through the phloem using water and metabolic energy.

Mass flow – simplified

The generally accepted hypothesis for how assimilates move within a plant is called the Mass Flow Hypothesis (also called Pressure Flow Hypothesis). Glucose, manufactured in the leaf during photosynthesis, is converted to sucrose (a dissacharide composed of a molecule of glucose attached to a molecule of fructose). Sucrose is then actively loaded into sieve tubes of the phloem (living cells). Movement of sucrose into the sieve cells of the sieve tube causes the water potential of these cells to become more negative. This more negative water potential creates a gradient which draws water from neighboring xylem cells into the sieve cells. This in turn creates a pressure. The phloem sap will begin to move along the sieve tube due to the pressure gradient caused by the water movement into the sieve cells where the sugar was initially loaded.

Once the sap reaches a sink (such as a developing fruit, dividing cells of a meristem, or storage cells in the root) sucrose molecules are actively unloaded from the sieve cells of the sieve tube to mesophyll cells in the sink tissue. As this happens, the osmotic potential of the sieve cells becomes less negative and water begins to leave the sieve cells of the sieve tube. The sucrose is used for metabolism, used to

synthesize cellulose or converted into starch for storage. Starch is 'nice' for storing energy in a carbohydrate because it has no effect on the osmotic properties of the cell in which it is located. The water that leaves the sieve tube eventually returns to the xylem for the long trip back to the leaf.

This is mass flow, simplified. In order for this incredible phenomenon to occur, some rather fascinating things must happen at the cellular and tissue level. Let's look at the phloem a little more closely.

The phloem

Phloem consists of: (i) parenchyma cells for storage; (ii) fibers for support; (iii) sieve cells (also called sieve elements) for transport; and (iv) companion cells for metabolic help. Sieve cells, which are relatively long and slender compared with other cells, are placed end to end forming sieve tubes, an extensive conduit in which to transport phloem sap. At the ends of each sieve cell are large-diameter, membrane-lined pores called sieve pores, collectively called a sieve plate. As sieve cells develop, much of their protoplasmic contents degenerate leaving a functioning plasmalemma with protoplasm containing only a few plastids, mitochondria, and some smooth endoplasmic reticulum near the cell wall. Thus a functioning sieve tube is a long series of connected sieve cells with functioning protoplasm with few organelles. In other words, it is full of phloem sap and that's about all.

If the sieve cell is pretty much an empty cell, how does it survive? That's where the companion cells come in. Each sieve cell has one or more companion cells intricately connected to it via plasmodesmata, and together they form the sieve cell–companion cell complex. Companion cells have many ribosomes, mitochondria, and rough endoplasmic reticulum along with a prominent nucleus. The companion cell performs the metabolism sieve cells need to survive. Without companion cells, sieve cells would die.

What happens if one of these long tubes is damaged? Phloem sieve cells have a very effective mechanism for plugging sieve plates if necessary. Sieve cells are under high turgor pressure. Any rapid loss of pressure in a sieve cell causes a specific phloem protein (P-protein) to quickly plug up the pores in the sieve plate. Callose, a carbohydrate, is also produced in response to wounding or high temperature stress to plug the pores. Once plugged, that sieve tube no longer functions in long-distance transport of assimilates. It can function in transport up to the point of plugging.

Phloem sap

Phloem sap is mostly water. A general description of the contents of phloem sap based on a number of species would include: (i) it is 10–12% dry matter; (ii) it has a pH of 8.0–8.5; and (iii) it has considerable solutes in it, mostly non-reducing sugars and also amides, amino acids, organic acids, potassium, and trace amounts of auxins, gibberellins, cytokinins, and ABA. These solutes generate an osmotic pressure of 1.2–1.8 MPa.

Carbohydrates transported in the phloem are non-reducing sugars where the reactive aldehyde or ketone group of the molecule has been reduced to an alcohol, usually mannitol or sorbitol, or combined with a similar group from another sugar to form an oligosaccharide. Sucrose is the most widely transported sugar. There are some exceptions. Sorbitol is the principal transport sugar in members of the family *Rosaceae* and stachyose is the predominant transported sugar in members of the *Cucurbitaceae*.

Phloem transport occurs in the range of 2.8–11 g/m^2 phloem/s. Phloem sap has been estimated to move at speeds of up to 56×10^{-5} m/s or 200 cm/h.

Mass flow – in depth

Translocation in the phloem is caused by pressure gradients, derived from differences in osmotic pressure due to active loading and unloading of solutes from sieve cells. Solutes are loaded into sieve cells at the source end (leaf) of the phloem. This causes the osmotic pressure of the sieve cell to become more negative, water enters the cell and increases the pressure within. The increased pressure forces the contents in the phloem to flow towards the area of lower pressure existing at the sink end of the phloem (fruit, meristem, storage roots). At the sink, the solutes are actively unloaded from the sieve cell, causing the osmotic pressure to be less negative, and water leaves the cell, thereby reducing the pressure.

The main question surrounding the Mass Flow Hypothesis is whether or not sufficient pressure exists within the phloem to make it work. Direct measurement of pressure in the phloem is difficult. However, indirect estimates support the hypothesis in this regard. Another important question is

whether or not the pressure gradient is steep enough from source to sink to generate the phloem flow observed. Estimates of the gradients between source–phloem–sink are sufficient to account for the flow observed.

The phloem has a tremendous capacity for transport. As previously noted, phloem transport normally occurs in the range of 2.8–11 g/m^2 phloem/s. A phloem transport rate of 305 g/m^2 sieve-tube area/s was observed in castor bean when the pressure at the sink end was removed by cutting the fruit pedicel and allowing flow to occur unobstructed. This observation indicates that crop productivity does not seem to be regulated by phloem transport but rather by loading and unloading of the phloem. Phloem has the capacity to transport a tremendous amount of assimilate.

Since movement of assimilate from source to sink seems to be primarily regulated by loading at the source and unloading at the sink, let's look at the two processes.

Phloem loading

Phloem loading occurs primarily in the leaves. To be exact, phloem loading is the process of photoassimilate transport from photosynthesizing mesophyll cells in the leaf to sieve cells in the phloem. Most loading into sieve cells occurs in the minor veins of a leaf while the transport occurs through major veins. Minor vein ends consist of a single xylem element, a few parenchyma cells and one or two sieve cells symplasmically connected to one to four companion cells.

Photoassimilates can travel from mesophyll cells to sieve cells via plasmodesmata or by exiting the mesophyll cell through the plasma membrane, travelling through the cell wall and then into a sieve element traversing its plasma membrane. The former is called symplasmic phloem loading and the latter is called apoplasmic phloem loading (Fig. 7.6). Which path sugar molecules take is still a source of debate. A broad generalization is that more primitive species utilize symplasmic phloem loading while more highly evolved species use apoplasmic phloem loading. Many herbaceous species and most crop plants belong to this latter group. Some species utilize both pathways.

With either apoplasmic or symplasmic loading, the mechanism for doing so must account for selective loading of solutes into sieve cells and the resulting elevated solute concentrations within the sieve cell–companion cell complex.

Symplasmic loading

Most plants that load assimilates symplasmically translocate between 20 and 80% of the sugars in a raffinose-related compound such as raffinose, stachyose, or verbascose. The general hypothesis is that sucrose diffuses from mesophyll or bundle sheath cells into companion cells via plasmodesmata. Once in companion cells, sucrose is enzymatically converted into oligosaccharides (raffinose and stachyose) which maintain a diffusion gradient for sucrose. Raffinose and stachyose are larger molecules than sucrose and are prevented from diffusing back into mesophyll or bundle sheath cells by size-excluding plasmodesmata. The raffinose and stachyose diffuse into sieve cells.

Apoplasmic loading

The apoplasmic loading model accomplishes selective concentration of solutes in sieve cells via an energy-dependent membrane transport pump. Genes have been identified which are selectively expressed in leaf phloem which encode this sucrose pump. Unfortunately not much is known about sucrose efflux out of mesophyll cells.

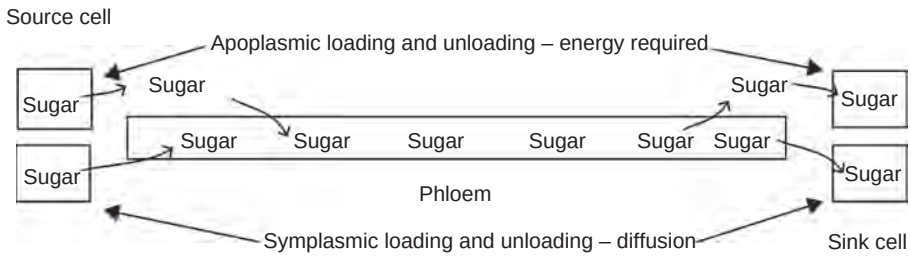

Fig. 7.6. The mass flow model of phloem transport: phloem loading and unloading.

Regulation of flow

When the source is limiting photoassimilate transport due to less-than-maximum synthesis at the leaf, changes in sink demand have no effect on the rate of transport. In other words, even if sink demand is high, during fruit growth for example, the flow in the phloem is controlled by the synthesis of sugar in the leaf. On the other hand, if the source is not limiting, any change in sink demand greatly changes export from the leaves. If demand is high in the sink, phloem flow will be high if there is sufficient assimilate being produced in the source. Sink regulation of transport from the source by sinks is likely via effects on the sucrose-loading membrane pumps in the leaf, most probably controlled by pressure changes in the phloem. The response to pressure would occur within minutes. Gibberellin applied to leaves leads to a rapid increase in photoassimilate loading into the phloem.

Phloem unloading

The final step in the phloem transport chain is unloading products of photosynthesis into the sink, or phloem unloading. Once at the sink, the transported photosynthates have one of three fates: (i) cellular catabolism in respiration; (ii) biosynthesis of components needed for growth and maintenance; and (iii) conversion into storage products that regulate transport among competing sinks. Phloem unloading is actually composed of two distinct components: (i) unloading from the sieve cell; and (ii) transport to the sink cell. While phloem loading occurs within a very specific framework, from mesophyll cells to sieve cells in a leaf, phloem unloading occurs over a much broader framework.

Unloading can occur in root or shoot apices, various cells of the stem or root, vegetative storage organs, or reproductive organs. Unloading also occurs in different cell types such as differentiating sieve cells, sieve cells lacking companion cells, or sieve cells with active companion cells. Cells into which the phloem is unloading may be dividing or expanding and the metabolism of these cells may vary from respiration to synthesis of components of growth, maintenance, or storage. In order to accommodate these various configurations for phloem unloading, a wide range of mechanisms of unloading exists.

Pathways of unloading

Unloading phloem solutes take one of three cellular pathways: (i) apoplasmic; (ii) symplasmic; or (iii) symplasmic–apoplasmic–symplasmic. Photoassimilates can be directly unloaded across the plasma membrane of sieve cells into the apoplasm of sink cells. This is apoplasmic unloading and it occurs especially in radial unloading to storage parenchyma cells in roots and stems. If the assimilate moves directly from cell to cell with no entrance into the apoplast, travelling through plasmodesmata, symplasmic unloading occurs. This is typical in apical meristems of shoots and roots as well as vegetative storage organs such as potato tubers. In some situations, the assimilate must leave the symplast, temporarily entering the apoplast, before re-entering the symplast as it is unloaded into sink tissue. Developing seeds demonstrate this type of unloading. There is an apoplasmic space separating symplasts of maternal and filial tissue in seeds which prevents totally symplasmic transport. Another example is the transport of assimilates from root tissue to mycorrhizas or from stem tissue to mistletoe.

Once unloaded, transport through the sink tissue is primarily symplasmic. Since symplasmic transport does not involve membrane transport, resistance to flow is much less in symplasmic transfer compared with apoplasmic transport.

In some situations, the mode of transport switches, especially in developing sinks such as fruit. The tomato is a good example. Early in fruit development phloem unloading is symplasmic. Sugars are converted into starch in sink cells, maintaining a pressure gradient for further phloem unloading. Later during fruit expansion, transport switches to an apoplasmic route. This is necessary because during expansion, sugars are not converted into starch. As such, osmotic pressure is generated in sink cells. In order to avoid shutting down phloem transport due to building pressure in sink cells, the phloem is 'separated' from the sink via the apoplasm.

Mechanism of unloading

Since the concentration of sugars in sieve cells is high, a concentration gradient exists that facilitates leakage of sugars into the apoplasm. Some of this sucrose is reloaded back into the sieve cell by membrane-bound pumps, thus the total amount of

sucrose unloaded via diffusion is determined by the concentration gradient inside and outside of the sieve cell as well as the activity of the membrane pumps.

Symplasmic transport occurs via cytoplasmic streaming and movement through plasmodesmata. The limiting phase of the process appears to be movement through plasmodesmata.

In the seed system where there is an apoplasmic barrier between two symplasmic steps of phloem unloading, the efflux of assimilate from the first symplasmic phase into the apoplasmic phase occurs via a sucrose–proton antiport membrane pump (sucrose out, proton in). Sucrose uptake from the apoplasmic phase into the second symplasmic phase occurs via a sucrose–proton symport membrane pump (sucrose in, proton in).

Fate of unloaded assimilates

Once unloaded, assimilates may be used for respiration, biosynthesis of components needed for growth and maintenance, or conversion into storage products. What happens to assimilates depends on the sink cell function. No matter what the specific function of the sink cell, part of the imported assimilate is used for respiration in cell maintenance. In growing cells, some of the imported assimilate is used for synthesis of cell materials. In general, approximately 40% of assimilates are used in respiration and 55% is used for growth materials. In mature cells, much of the imported assimilate is stored (about 80%) as insoluble starch in vacuoles or amyloplasts or sugar compartmentalized in the vacuole. Stored sugars are usually sucrose, hexoses, or fructans (short-chain polymers of fructose). Sucrose and hexoses are particularly important in fruit and vegetable quality, while fructans are important in pasture forage quality or remobilized for storage in the grain. The storage carbohydrate may change during development. For example, young fruit of tomato store starch while maturing fruit store hexoses, and convert stored starch to hexoses. The rest is needed for maintenance respiration. Storage may be short (hours or perhaps days) or long term (months to perhaps years).

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8 Light Energy and Plant Function

Through the amazing process of photosynthesis, light energy from the sun is captured and stored as carbohydrates, proteins, and fats. We depend on these products of photosynthesis for food, fuel, shelter, clothing, and animal feed. In addition to being the driving force of photosynthesis, light also orchestrates the growth and development of most plant species. Understanding why something as simple as depth of planting at seeding is so important to production success relies on understanding the physics and biology of light. In this chapter the amazing involvement of light in plant growth and development will be examined.

The Physics of Light

Humans have always wondered what light is and how it travels. We know that light is energy, but we really don't know how it moves. There are two major theories of how light travels. One theory proposes that light travels as discrete packets of energy called photons. The other suggests that light travels as waves, just as energy in water travels as waves. Both theories are correct. Light behaves as a wave in some ways and as a particle in others. For plant scientists, it doesn't matter whether light travels as a wave or as a particle. We only need to know how much light there is (intensity or level) and its quality (wavelength).

Light intensity refers to the amount of light at the source while light level refers to the amount of light when it reaches an object. This is an important distinction when discussing light and plants. The intensity of a light source ultimately determines the level when plants intercept the light. We can't control the intensity of our most important light source, the sun, but we can control its level intercepted by plants. We alter plant light levels through pruning, plant spacing, and the use of shading technologies such as lathe houses or shade cloths. We can control both factors when dealing with artificial lighting by bulb selection and altering the distance between plants and the light source.

Light Quality

Light quality is described by its wavelength, measured in nanometers (nm). While many discussions surrounding light often only consider visible electromagnetic radiation, we need to consider the entire electromagnetic spectrum from ultraviolet (UV) through to infrared. Wavelengths beyond these endpoints do not normally impact plant growth and development. Table 8.1 summarizes the wavelengths, color, and importance in horticulture for light from UV to infrared.

Light Quantity

Light quantity is measured as the instantaneous amount of light hitting a unit area per unit time. The units of measurement differ for different types of light. Light we see is measured in foot candles and lux. Total solar radiation is measured in watts (W), and light available to plants for photosynthesis is measured in micromoles (μmol). Quantities of light are measured as particles called photons or quanta. Each photon has a very small amount of energy associated with it, thus we measure photons in units of moles (mol). One mole is equal to 6.02×10^{23} photons. A micromole (μmol) is one-millionth of a mole, or 602,000,000,000,000,000 photons.

An instantaneous measurement of light is not very useful for assessing its impact on plants. A measurement of the total quantity of light at a particular wavelength over time is much more desirable. One such measurement is the daily light integral (DLI) and is the daily total of photosynthetically active radiation (PAR, 400–700 nm) measured in moles per square meter per day ($mol/m^2/day$).

Table 8.1. Impacts on plant growth and development of wavelengths particularly important to horticulturists.

Wavelength (nm)	Color	Impacts on horticulture
280–315	UV	May bleach leaves, may cause sunburn, particularly on fruit, may induce genetic mutation
315–400	UV – blue	Important in photoperiodic responses, some absorption by chlorophyll, thus may influence photosynthesis, inhibits cell elongation, can cause sunburn
400–520	Blue	Photosynthesis
520–610	Green	Mostly reflected by plants
610–750	Red	Photosynthesis and photoperiodism
750–1000	Far-red	Stimulates cell elongation, influences flowering and germination
1000+	Infrared – heat	Heat

In general the middle latitudes receive about 9 mol/day PAR on a sunny winter day, and only about 3 mol/day PAR if it is cloudy. In the summertime, it's about 26 and 12 mol/day for sunny and cloudy days, respectively (Tibbitts, 1994). DLI measurements summed over the season can help in decisions regarding cultivar suitability for specific locations, especially for out-of-season production.

Light Meters

Foot candle or lux meters

Foot candle or lux meters are useless for measuring light in relation to plant productivity. These meters measure light much like the human eye perceives light, mostly in the 500–600 nm range. Much of this is green light, which is not really used by plants but rather is reflected back into space.

PAR/quantum light meter

PAR is radiation effective for driving photosynthesis. Light between 400 and 700 nm is considered PAR. This is the light level most often measured by horticulturists as it provides a good idea of how much light is generally available for plant growth and development. It is often called quantum light and the meter is called a quantum light meter. PAR is measured as the number of moles of light photons striking a surface (in this case the light sensor surface) per unit area per unit time.

Solar radiation meter

A solar radiation meter measures radiation in a very broad spectrum from 300 to 1100 nm. Measurements of this type of radiation are important for estimating evapotranspiration and scheduling irrigation.

UV meter

UV meters are useful in measuring the effectiveness of UV filtering materials that might be used in high tunnel or greenhouse covers. Most UV meters measure radiation in the 250–400 nm range. Meters are available for measuring specific zones within this range.

Red/far-red meter

This type of meter calculates the red:far-red ratio of light by measuring red (660–680 nm) and far-red (720–740 nm) light. These wavelengths are important in phytochrome-mediated responses. These meters are particularly useful for determining the influence of leaf canopy on this ratio as measurements can be made in a number of canopy locations and mapped. Decreasing red:far-red ratios can lead to unwanted stem elongation and 'stretching' of plants. Thus, knowledge of red:far-red ratios is important in determining plant spacing, particularly in greenhouse production which can influence the need for anti-elongation growth regulator applications.

Light Quality and Artificial Lighting

Light quality and quantity are critical choices in artificial lighting situations such as indoor or greenhouse production. Light quantity can be regulated by the number of lamps used and their distance from plant canopies. Light quality is determined by bulb selection.

Lamp technology

There are a number of technologies available for generating light energy from electricity (Dakin, 1994). Many of them have been employed at one time or another for use in horticultural production

systems. After basic spectral requirements are met, the initial cost of the luminaire (the entire lighting fixture consisting of bulb, ballast, and reflector), operating and maintenance costs as well as heat generation must be considered.

Incandescent bulbs

An incandescent bulb has a tungsten filament which heats up to 2800 K as electricity passes through it. Some of the energy is radiated as light while much of it is released as heat. Ultimately, the filament 'burns out' and the bulb must be replaced.

Discharge light sources

In discharge light sources, an electric current passes between two electrodes and heats gaseous plasma contained in a tube made of glass called an arctube. Discharge light sources don't burn out like incandescent bulbs, since they have no filament. They slowly decline due to changes in the electrodes and walls of the arctube. The differences among the discharge types are due to differences in arctube composition, wall temperature, and electrode gap distance. Generally there are two gases involved in each type of discharge source, one is the dominant gas and the other is responsible for radiation. When an electric current passes between the two electrodes, the dominant gas in the tube is ionized, gaining energy from the electric current. When these excited ions collide with atoms in the radiating gas, some of the radiating gas electrons become excited, eventually releasing the energy as photons of light.

FLUORESCENT BULBS Nearly everyone is familiar with the fluorescent discharge light source. The dominant gas is argon (Ar) while the radiating gas is mercury (Hg). This type of lamp is often called a low pressure Hg-Ar discharge lamp. This is also the type of lamp in many neon signs. Low pressure means that the collision rates between gas atoms are relatively low. The radiation emitted is mostly UV (254 nm). Visible light is produced when the UV radiation strikes a phosphor coating on the inside of the arctube wall. The discharge is efficient at creating UV radiation, but the conversion to visible light is inefficient (Langhans, 1994).

HIGH PRESSURE MERCURY LAMPS High pressure discharge lamps are smaller than fluorescent tubes and operate at higher power, temperature, and pressure (Fig. 8.1). The center of the discharge is nearly 5000 K, almost as hot as the sun.

High pressure mercury lamps have Hg as the dominant and radiating gas. While much of the radiation is in the UV range, mercury lamps discharge visible light mostly around 405, 435, and 545 nm.

High pressure metal halide (MH) lamps are similar to high pressure mercury lamps. In MH lamps, there are small amounts of sodium iodide (NaI) and scandium iodide (ScI_3). When the lamp is operating, these salts are molten condensates on the arctube walls with low concentrations of their vapors in the arctube gas. The Na and Sc atoms have lower energy levels than the Hg and radiate more readily than the Hg in the arctube gas. Most of the energy of the Na and Sc atoms is in the visible range. Thus the MH lamp has a higher visible efficiency (more visible light per unit of energy used) than the high pressure mercury lamp.

Both the high pressure mercury tube and the MH lamp utilize high pressure Hg in a fused quartz arctube. Greater visible efficiency in discharge lamps can be obtained by increasing the operating temperature, however, the useful life of the lamp decreases as operating temperature increases. Additionally, in these two lamp types, the high temperature limit for the quartz tube is about 900°C.

High pressure sodium lamps are similar to MH lamps in that they rely on radiation from Na atoms in the presence of Hg. In high pressure sodium lamps, higher amounts of Na in the vapor phase are achieved by using elemental Na rather than

Fig. 8.1. A high pressure discharge luminaire for supplemental greenhouse lighting.

NaI. The arctube wall is made of Al_2O_3 rather than quartz and can withstand temperatures up to 1150°C resulting in higher visible efficiency (Langhans, 1994).

LED LIGHTS Light emitting diodes (LED) are solid-state light-generating fixtures which will probably become one of the biggest advancements in protected plant culture in many years (Bula *et al.*, 1994; Morrow, 2008). LEDs produce light via an electrical current flowing through a solid material with *p-n* junctions. A *p-n* junction is the junction of a *n-type* electron donating semiconductor and the a *p-type* electron accepting semiconductor. The original LEDs were expensive and generated only red (660 nm) light. Current LED structures combine LED units which generate specific wavelengths into a combined lighting structure called an array, analogous to the luminaire of conventional lighting systems.

LEDs are desirable as light sources in horticulture for many reasons: (i) they have a long life; (ii) they are small and rugged; (iii) they have low voltage requirements; and (iv) they do not generate excessive heat. Another unique aspect of LED lights is that wavelength emission can be tailor designed by altering the specific LEDs used in the structure. Programmed wavelengths would be useful for photomorphological manipulation. In addition, LEDs are much safer than conventional lighting fixtures: LEDs do not have glass bulbs with high temperatures that are dangerous and easily broken and they do not contain Hg.

LED arrays are particularly well suited for research applications. In addition, they generate minimal heat and have a low profile, making them ideal for shelving applications often used in tissue culture. Ultimately, LEDs are likely to become widely used in greenhouse applications for supplemental lighting and photoperiod manipulation. The major limitation to their use in horticulture is purchase cost. Another limitation is low output at wavelengths needed for optimum plant growth and development. Both limitations have been decreasing over the last 10 years and will probably continue to do so.

SULFUR LAMPS Sulfur lamps have been considered as a source for lighting in horticulture (MacLennan *et al.*, 1994). Sulfur bulbs work somewhat like other high intensity discharge (HID) lamps, utilizing plasma inside a glass bulb which emits light when heated. The source of energy in a sulfur bulb is microwave energy generated by a magnetron similar to those found in microwave ovens. Electrodes are unnecessary. The sulfur lamp has no discernible spectral shift over the lamps lifetime (10,000 h). They have no large spikes at any wavelength and are very efficient, capable of providing 2000–6000 μmol/m^2/s PAR. Sulfur lamps have not become widely used in horticulture.

Horticultural applications of different light sources

Fluorescent

Fluorescent bulbs have been used extensively in growth chambers and growth rooms. Growth chambers are relatively small boxes equipped with temperature controls and light fixtures to study plant growth and development under controlled simulated outdoor conditions. Growth rooms are similar but much larger. While the spectral quality and intensity of sunlight is difficult to mimic, cool white fluorescent bulbs have become the standard in growth chambers and rooms for good reasons. Cool white fluorescent bulbs have the greatest mole output of photosynthetic photon flux (PPF, light for photosynthesis) of all fluorescent bulb types with an acceptable spectral distribution. Additionally, light levels of 600 μmol/m^2/s can be achieved. However, fluorescent bulbs have a relatively short lamp life (5000–10,000 h) concomitant with a rapid decay in spectral quality and quantity.

Use of fluorescent bulbs in the greenhouse is common. They are relatively inexpensive, readily available and easy to install. Their main liabilities are the same as described for growth chambers and rooms. In addition, lamps can cause excessive shade on benches and lamps must be 1 m or closer to plants in order to provide sufficient PPF levels.

High intensity discharge (HID)

HID lamps include high pressure mercury lamps, high pressure MH lamps, and high pressure sodium lamps. All three types can be used in growth chambers, growth rooms, or greenhouses. If light levels above about 500–1500 μmol/m^2/s are required, HID lamps must be used. HID lamps have a long lamp life (about 30,000 h for high pressure sodium lamps and 15,000 for MH lamps) along with a high efficiency of PPF output. High pressure

sodium lamps produce more PPF μmol/m²/s per unit electricity, but MH lamps have a better spectral distribution. The spectra of MH lamps are satisfactory for plant growth while high pressure sodium lamps are satisfactory only at PPF levels >700 μmol/m²/s. At lower PPF levels, the spectra may be deficient in blue for many species.

At high PPF levels in growth chambers or rooms, heat can be a problem with HID lamps and an air- or water-cooled barrier between the light units and the plants must be installed to catch and remove the heat generated. Because of heat generated at any PPF level, plants must not be closer than about 1 m from the lamps.

In the greenhouse, high pressure sodium lamps are the best choice because bulbs will last around 30,000 h and they have the best efficiency of PPF per unit electricity. The lower levels of blue light in high pressure sodium lamps compared with MH lamps are adequately supplemented by sunlight. When installing HID lamps in a greenhouse limit their installation to 200 μmol/m²/s or ballast heat and luminaire shade will be excessive.

Guidelines for supplemental greenhouse lighting

Most horticulture relies on sunlight for production. With the exception of high tunnel production for season extension, out-of-season production relies on supplemental lighting in the greenhouse to maximize productivity and crop quality (Dietzer *et al.*, 1994; Geiger and Noname, 1994). To develop a framework for lighting requirements, consider the following. Most species will grow productively with a daily light integral of 26 mol/m²/day. This level corresponds to an instantaneous irradiance of 300 μmol/m²/s for 24 h or 600 μmol/m²/s for 12 h. As a point of reference, the summer daily irradiance maximum is around 62 mol/m² in Phoenix, Arizona and in the winter the minimum irradiance is 8 mol/m² at Madison, Wisconsin. The average annual daily irradiance is about 26 mol/m² in Madison, Wisconsin and Washington, DC. At midday in the summertime, the highest solar irradiance is around 2000 μmol/m²/s.

In most greenhouse situations, a total DLI of 26 mol/m² is sufficient for most crops. This total is derived from sunlight plus supplemental irradiance. Lamps should provide a maximum of 200 μmol/m²/s, as levels greater than this add too much heat to the greenhouse and the number of luminaires required would shade plants.

Spectral comparison of horticultural light sources

Table 8.2 allows for a quick comparison of spectral quality among common horticultural light sources. All values were normalized to 100 μmol/m²/s of PAR (400–700 nm) and reflect the percentage of radiated light at a particular wavelength.

How Do Plants Measure Light?

We previously described the different meters used to measure light. Plants in a sense have their own built in light meters called photoreceptors. Photoreceptors are molecules that capture light energy and transform the light energy for utilization by the plant. The energy can be transformed to store energy, as in photosynthesis which is accomplished with the photoreceptor chlorophyll and

Table 8.2. Spectral comparison of common horticultural light sources (adapted from Dietzer, 1994).

Source	UV-B (250–350)	UV-A (350–400)	Blue (400–500)	Green (500–600)	Red (600–700)	Far-red (700–750)
Sunlight	2.88	6.21	29.16	35.20	35.64	17
Incandescent (100 W)	0	0.47	7.52	28.49	63.98	47
Cool white	0.3	1.11	24.85	52.59	22.56	1.4
Gro-Lux	0.16	3.72	24.36	20.22	50.42	1.01
Low pressure sodium	0.03	0.15	0.12	99.33	0.54	0.04
High pressure sodium	0.17	0.53	6.52	56.57	36.91	4.00
Metal halide (MH)	0.66	6.71	20.38	55.82	24.1	4.00
Cool white plus incandescent (100 W) 3:1 ratio	0.02	1.03	22.63	49.22	28.15	8.00

accessory pigments. The energy can also be transformed into a signal to elicit a physiological response. The photoreceptors responsible for this kind of light perception are phototropins, cryptochromes, and phytochrome (Smith, 1982, 1994, 2000; Cashmore *et al*., 1999; Briggs *et al*., 2001; Lin *et al*., 2001; Bouly *et al*., 2007).

While we classify the photoreceptors with specific light absorbance and physiological function, keep in mind that these photoreceptors often overlap in function. For example cryptochromes and phytochromes overlap in regulating such processes as: (i) inhibition of hypocotyl elongation; (ii) anthocyanin production; (iii) sensitivity of flowering to photoperiod; and (iv) entrance into circadian rhythms.

Phototropins

Phototropins (known as phot1 and phot2) are responsible for sensing blue (390–500 nm) and UV-A (320–390 nm) light which elicit a number of different physiological responses in plants (Briggs *et al*., 2001). These responses are generally movement based rather than a developmental response. The major response plants have to blue or UV-A light include: (i) the phototropic response (bending towards or away from a light source); (ii) chloroplast migration in leaf cells; and (iii) solar tracking of leaves of some species. Phototropins are also likely to be involved in the inhibition of stem elongation induced by blue light and blue-light-induced calcium uptake. While phot1 and phot2 are distinct and separate photoreceptors and phot2 only functions at high light intensities, there is considerable overlap in their functions. Neither are photoreversable.

Phototropins, particularly phot1, mediate phototropism, a plant's directional bending in response to light (Whippo and Hangarter, 2006; Holland *et al*., 2009). Positive phototropism is bending towards the light, as in shoot tips, while negative phototropism is bending away from the light, as in root tips. The bending response is due to unequal growth on opposite sides of the affected organ. The phototropic response is usually limited to differentiating tissue only. Mature tissue will not usually show a phototropic response. This is generally due to a loss in cell wall elasticity and a hardening of cell walls which makes them resistant to change. Hardened cell walls prevent uneven cell wall expansion which causes the bending of the organ involved.

Phototropins also mediate chloroplast migration within the cell in response to light levels. Under low light conditions, both phot1and phot2 induce chloroplast migration within the cell to minimize shading and maximize light interception. Under high light, chloroplasts are rearranged to minimize light interception to avoid photodamage. This high light response seems to be controlled exclusively by phot2. The molecular form of the molecule, phot1 or phot2, is determined by the amino acid sequence.

Phototropin molecules (either phot1 or phot2) exist as one of three forms: LOVD447, LOVL660 or LOVS390, depending on light conditions. LOVD447 is the dark form, ready to absorb blue light. The absorption of a single photon of blue light converts LOVD447 to the energized, intermediate form, LOVL660, which can absorb red light. This intermediate form rapidly decays to LOVS390 in darkness or after absorbing a second near UV photon. LOVS390 is the lit form which is responsible for signaling physiological responses.

Another interesting characteristic of phototropins is their movement within the cell in response to light signals. When LOVS390 is formed in response to blue light hitting LOVD447, the entire molecule moves within the cell. For example most phot1 (LOVD447 form) is normally associated with the plasma membrane in the dark. Once lit by blue light, some of the phot1 (now in the LOVS390 form) moves intracellularly. The actual movement of the molecule is a response to blue light. Similar movements have been observed for phot2.

Cryptochromes

Another group of blue and UV-A photoreceptors are the cryptochromes (Cashmore *et al*., 1999; Bouly *et al*., 2007). While phototropins regulate movement-based responses to blue and UV-A light, cryptochromes regulate developmental responses to blue and UV-A light. Additionally, cryptochromes interact significantly with phytochromes in these regulations of development. Cryptochromes are not reverseable photoreceptors.

There are a number of cryptochromes with those of *Arabidopsis*, CRY1 and CRY2, receiving the greatest attention for research. Cryptochromes regulate plant developmental responses to blue and UV-A light. Some developmental responses mediated by cryptochromes include: (i) hypocotyl elongation; (ii) entrainment in circadian rhythms especially those related to phytochrome-mediated

flowering responses; (iii) cotyledon expansion; (iv) anthocyanin production; (v) inhibition of stem elongation; (vi) stimulation of leaf expansion; and (vii) regulation of gene expression. CRY1 functions mainly under high light conditions while CRY2 functions mainly under low light levels. CRY2 is rapidly degraded at high intensities of blue light. In the light, most of the CRY1 is in the cytoplasm while the CRY2 is in the nucleus. CRY1 may be imported to the nucleus in the dark, but is quickly exported to the cytoplasm in the light.

CRY2 particularly interacts with phytochrome in photoperiod measurement. *Arabidopsis* is a long-day plant that flowers in response to short daily dark periods. Continuous illumination with blue or far-red light promotes flowering. Continuous illumination with red light inhibits flowering. CRY2 seems to promote flowering by interfering with the inhibition of flowering caused by an abundance of phytochrome far-red (P_{fr}) under red-light conditions and also by some unknown mechanism directly promoted by blue light.

As a seed germinates it undergoes a process called de-etiolation in response to exposure to light, blue light in particular. De-etiolation includes inhibition of stem elongation, enhanced leaf expansion, stimulation of chloroplast development and changes in gene expression. Cryptochromes trigger anion channels in the plasma membrane which results in depolarization of the membrane and a subsequent reduction in cell expansion.

Cryptochrome, at least CRY1, appears to exist in at least two interconvertable forms. Inactive CRY1 accumulates in the dark. Exposure to blue light reduces the inactive CRY1 to an active form. The activation can be inhibited with green light, and as such, green light can inhibit plant responses to blue light. This occurs in nature in plants growing under the canopy of another species. The low-growing plants are subject to light rich in green wavelengths. This helps explain why plants often elongate in response to shading.

Mystery blue and green light receptors

Responses to photoreceptor stimulation follow a very precise curve when comparing response to wavelength exposure. This curve is called an action spectrum and each known photoreceptor has a specific curve for activity. If a plant response is controlled by a particular photoreceptor, the response should closely follow that photoreceptor's action spectrum.

There are several examples of plant responses to blue light that do not seem to be under the control of any known photoreceptor. Blue-light regulation of stomatal aperture is controlled by an unknown photoreceptor. Zeaxanthin, a xanthophyll pigment, may be involved as a light receptor in stomatal function. The action spectrum driving its synthesis is similar to that regulating stomatal opening. Another 'mystery' blue-light receptor regulates leaf-base folding in *Oxalis*. The action spectrum for this phenomenon does not match any known photoreceptor.

Green light can affect hypocotyl elongation and leaflet folding in the sensitive plant (*Albizzia julibrissin*) (Folta and Maruhnich, 2007). Green light can also induce changes in mRNA transcription in plastids. Both of these responses appear to be controlled by an unknown photoreceptor.

Phytochrome

Phytochrome is the most widely studied photoreceptor in plants (Smith, 1982, 1994, 2000). It is responsible for photoperiodic responses in growth and development. Photoperiodism is the ability of a plant to measure daylength as a relative amount of light to darkness. Most photoperiodic responses are controlled by length of the dark period (nyctoperiod), not the light period. Photomorphogenesis is a plant's developmental response to night length.

There are a number of different phytochromes in plants with five different phytochromes (A, B, C, D, and E) isolated from the model plant *Arabidopsis*. It is likely that most higher plants have the same or similar phytochromes. Phytochrome is localized in the cytoplasm of the cell, the nucleus, and in plastids. Not all cells contain the same amount of phytochrome. In the epidermis, most of the phytochrome is in the guard cells of the stomata.

Phytochrome occurs in plants in two basic forms: (i) phytochrome red (P_r) which absorbs red light (660 nm); and (ii) phytochrome far-red (P_{fr}) which absorbs far-red light (730 nm). When a molecule of P_r absorbs a photon of red light, it is immediately transformed into P_{fr}. P_{fr} is very unstable and degrades slowly in the dark back to P_r. Additionally, P_{fr} is converted to P_r upon exposure to far-red light (730 nm).

The phytochrome molecule consists of a protein which binds to a chromophore, or light-absorbent molecule. The protein is encoded in nuclear DNA which is transcribed in the nucleus and translated

on ribosomes in the cytoplasm. The chromophore is manufactured in plastids. The two components are brought together to form a phytochrome molecule in the cytoplasm.

This assembled molecule of phytochrome is a molecule of P_r. When the molecule is exposed to red light, its structure is modified by the energy in the red light, and the phytochrome molecule is transformed to the P_{fr} form. In addition, the P_{fr} molecule autophosphorylates. The phytochrome is now the physiologically active P_{fr}. The amount of P_{fr} remaining after the dark period determines whether or not a photoperiodic response is observed.

Photoperiodic responses controlled by phytochrome are often classified as inductance responses or high irradiance responses (HIR) based on the photon flux density required to initiate the response and the timing of irradiance.

The inductance responses are further classified as very low fluence responses (VLFR) and low fluence responses (LFR). Both VLFR and LFR reactions can occur with only a few seconds of irradiation and the response will occur even if the seed or plant is returned to darkness. VLFR responses are mediated by phytochrome A, activated by red light, and are not reversible by exposure to far-red light. Very weak pulses of light equivalent to one to three firefly flashes are all that are required for VLFR to occur.

LFR responses are controlled by phytochrome B, regulated by red and far-red light and are reversible. A few seconds to a few minutes of light equivalent to a common flashlight are needed to induce this type of response. Many of the typical responses attributed to phytochrome control are LFR responses.

HIR responses are not reversible and are controlled by phytochromes A and B. They require prolonged high intensity irradiation and the response is in proportion to the irradiance received by the plant. If the tissue is etiolated, as in a germinating seedling, far-red and blue light are effective in mediating the response. If green tissue is involved, red light is active. HIR responses generally take minutes to hours of high fluence irradiation to occur. Two of the typical HIR responses are: (i) anthocyanin production; and (ii) inhibition of hypocotyl elongation.

A useful parameter to describe light quality, especially with respect to phytochrome-mediated responses, is to describe the ratio of red to far-red light. It is important to remember when discussing this ratio, the ratio describes the ratio of light, not the ratio of the phytochrome forms. A high R:FR light ratio (high red light) would yield a low P_r:P_{fr} ratio (high P_{fr}) while a low R:FR light ratio (high far-red light) would yield a high P_r:P_{fr} ratio (high P_r).

Daylight contains about equal amounts of red and far-red light (approximate red:far-red of 1:1.2) when the sun is more than 10° above the horizon. A small but detectable change in the red to far-red light ratio of sunlight occurs when the sun is less than 10° above the horizon (dawn and dusk). With the sun less than 10° above the horizon, sunlight has a longer path through the earth's atmosphere leading to greater light absorption and scattering. The sunlight spectrum at dawn and dusk is enriched in the blue and far-red regions, and poor in the orange-red regions. Thus, at dawn or dusk, the R:FR ratio is around 0.7–0.8.

In addition to sun angle, vegetation itself affects the relative amounts of red and far-red light. Vegetation absorbs red light but not much far-red light. Consequently the R:FR light ratio in canopies is often very low (high levels of far-red light) in the order of 0.09–0.7. The higher far-red light leads to higher levels of P_r which promotes etiolation (or conversely P_{fr} inhibits etiolation). Thus shoots will elongate more to effectively compete for available light as the canopy becomes more dense.

Plants enter the dark period with a certain P_{fr}:P_r. Any photoperiodic response will be governed by the amount of P_{fr} remaining relative to P_r at the end of the dark period. Long-day responses require a short dark period. This leads to a high P_{fr}:P_r ratio at the end of the dark cycle. Short-day responses need a long dark period leading to a low ratio of P_{fr}:P_r after the dark period.

Photoperiod, Phytochrome, and Plant Growth

True photoperiodic responses

In discussing plant developmental responses to daylength it is important to distinguish between responses attributable to the daily light period and those attributed to the daily dark period. This distinction is particularly important for long-day responses. Is the observed response due to a long period of photosynthesis or is it due to a high level of P_{fr} at the end of the dark period? The former does not imply a photoperiod response, but rather a photosynthetic response. The latter suggests a true photoperiodic response. But how can we separate the two?

The night interruption (NI) effect

A long-used method for separating photosynthetic and photoperiodic responses is called the 'night interruption (NI) technique' (Fig. 8.2). Plants are grown under three different conditions: (i) a short day (SD) (typically 8 h light with 16 h darkness); (ii) a long day (LD) (16 h light, 8 h darkness); and (iii) a photoperiodically long day (also called the NI treatment) (8 h light, 16 h dark with the dark period interrupted briefly, for as little as 1 min, in the middle with low-level red light). The plant response in question is compared among the three conditions. If the NI treatment produces a response much like the SD treatment, the response is due to photosynthesis differences between long and short days. If the NI response mimics that observed under long days the response is likely to be a true photoperiodic response. If the response is qualitatively but not quantitatively similar to long day, the response is likely to be due to both photosynthesis and photoperiod. A good example of this is the runnering response of photoperiod-sensitive strawberry (*Fragaria* × *ananassa*) cultivars. These cultivars will not produce runners under SD conditions. Under LDs, they produce runners profusely. NI treatment causes runner production, but not to the extent LD does. Clearly, the runnering response is a qualitatively true photoperiod response to short nights, but a quantitative response to length of the light period.

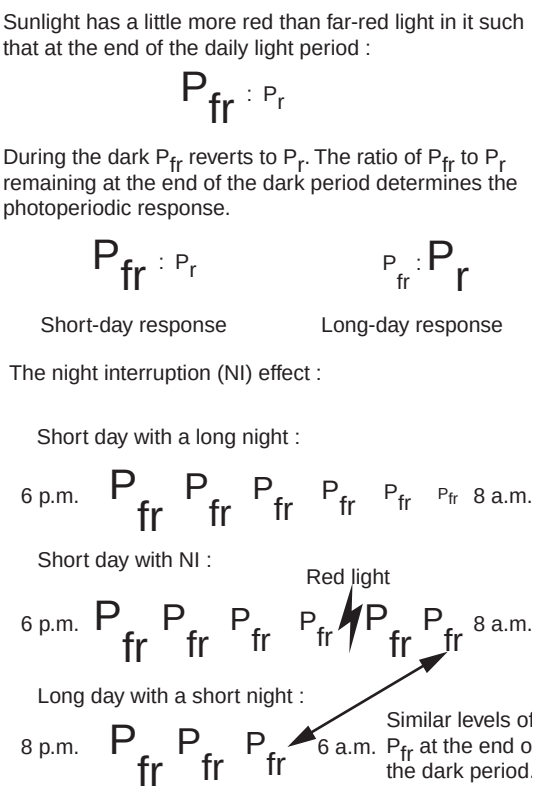


Fig. 8.2. Schematic representation of phytochrome conversions during night interruption (NI) with red light. (Lightning symbol courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science, ian.umces.edu/symbols/.)

Physiology of the NI effect

The physiological mechanism for the NI effect is simple and elegant. Under SD, there is little P_{fr} remaining at the end of the dark period. Under LD, there is abundant P_{fr} remaining at the end of the dark period. With the NI treatment, P_{fr} levels decline until the interruption treatment with red light converts all P_r back into P_{fr}. It thereby resets the P_{fr} level to what it would be at the start of the dark period. Since the LD dark period and the second half of the NI dark period are both 8 h, the level of P_{fr} at the end are similar. If the response is due to P_{fr} level (and hence a true photoperiodic response to the length of the dark period) it will be similar under LD and NI. The key to achieving the photoperiodic result anticipated with the NI treatment is using red light in the middle of the dark period. No other color and no other timing will work.

This NI treatment is useful in out-of-season production where a long-day response is desired under short-day conditions but the expense of supplemental daylength extension is not acceptable. The response desired must be a true photoperiodic response. A simple string of low wattage incandescent light bulbs will work (Fig. 8.3). A standard NI treatment in greenhouse production utilizes 15 W incandescent bulbs lit for 3 h in the middle of the dark period. The 3 h interruption is to ensure adequate conversion of P_r to P_{fr} with the incandescent bulbs.

Some responses to phytochrome

Many horticultural crops depend on photoperiodic signals for induction of a specific growth process directly related to harvest. The most notable and most widely studied process is flowering. Other processes include: (i) seed germination; (ii) de-etiolation of seedlings; (iii) tuber formation in potatoes; and

Fig. 8.3. A string of low wattage incandescent bulbs used for inducing the NI effect in fall-grown (New Jersey, USA) high tunnel strawberries (*Fragaria* × *ananassa*).

(iv) bulb formation in onions. Another photoperiodically controlled process important for propagation rather than harvest is stolon production in strawberry.

Seed germination

Seed germination is at least partially controlled by phytochrome in many species. Most species exhibit a classical P_r/P_{fr} reversibly reaction with exposure to red light and accumulation of P_{fr} leading to germination which is a LFR mode of phytochrome action.

A classic example of this is found in lettuce (*Lactuca sativa*). The photoperiodic control of germination is so strong in lettuce that germination of lettuce seed after exposure to red and far-red light is a classic laboratory experiment for many plant physiology classes. Seed germination of lettuce requires the presence of an adequate amount of P_{fr}. P_r is synthesized by plant tissues, including germinating seeds, and is converted to P_{fr} by exposure to red light. In order to germinate, lettuce seed must be exposed to red light, thus shallow coverage is essential for good stands. Many lettuce cultivars are primed before sale, and during the priming process the photoperiod requirement is met as well as any temperature treatment to break thermodormancy. Seed coverage is not such a big issue for primed lettuce seed.

The absolute requirement for red light is readily illustrated in a laboratory setting by imbibing lettuce seed in the dark and then exposing them to either red or far-red light, or cycles of red and far-red light. Only lettuce seed with a final exposure to red light will germinate. Those exposed to far-red light will not, since most of the P_{fr} is converted to P_r with the far-red light exposure.

Many seeds that are imbibed in darkness express the VLFR mode of phytochrome action. They remain dormant and are extremely sensitive to light. They will germinate only after light exposure. In seeds that can germinate in complete darkness, germination can be inhibited by exposure to prolonged far-red light, which is probably a reflection of the HIR type of the phytochrome response.

Seedling de-etiolation

As a seed germinates, the seedling is normally elongated with closed and unexpanded cotyledons that lack photosynthetic capacity. Once moved to the light, shoot growth is inhibited, cotyledons expand and the photosynthetic machinery revs up. All of this is in response to red light perception by phytochromes A and B, and blue light perception by cryptochrome and phytochrome A.

Shade avoidance

Plants have a unique ability to modify their architecture and reproductive strategy in response to shading, helping them avoid competition for light under crowded conditions. When a shade-intolerant species detects shading, it will initate internode and/or petiole elongation, increased apical dominance, retarded leaf development or accelerated flowering. All of these mechanisms are an attempt to elevate leaves to a better light environment, and to ensure seed production under marginal conditions. Many times the responses begin long before shading actually occurs due to far-red light reflecting off leaves of nearby neighbors. These physiological responses to shading are mediated through phytochrome, predominantly phytochromes B, D and E.

Flowering

Plant development usually proceeds along a fairly well-defined pathway from seed germination, through

vegetative growth, flowering, fruiting, senescence, and finally death. In some species the entire process occurs in several weeks while others live for years, cycling between vegetative growth, flowering, and fruiting many times before entering senescence leading to death. Many species rely on environmental cues for switching from one phase of growth to another, especially the transition from vegetative growth to flowering. One major environmental signal for undergoing the transition from vegetative growth to sexual reproduction is photoperiod.

There are three photoperiod types of plants with respect to flowering: (i) short-day; (ii) long-day; and (iii) day-neutral. Short-day plants begin the flowering process when days are as short or shorter than a given critical daylength. Long-day plants respond when the daylength is as long as or longer than a critical photoperiod. Day-neutral plants are not affected by daylength.

Remember, since flowering is a true photoperiodic response, the length of the dark period (skotoperiod, nyctoperiod) regulates flowering, not the daylength. The critical daylength is defined for each species through observation or research. It is often useful to determine not only the critical photoperiod but also the number of light/dark cycles need for floral induction. Generally short-day plants require fewer cycles for floral induction if days are significantly shorter than the critical photoperiod. Similarly, long-day plants require fewer cycles if the daylength is significantly longer than the critical photoperiod.

In addition, the critical photoperiod and number of required cycles can be affected by temperature. As a general rule of thumb, relatively cool temperatures can substitute for part of a short-day requirement and relatively warm temperatures can substitute for some of the long-day cycles. Generally fewer inductive cycles are needed when the photoperiodic requirement is substituted with temperature exposure. When considering flower induction in short-day plants, a minimal daylength is required for adequate photosynthesis.

While we often categorize species as long-day, short-day, or day-neutral, some species may include more than one photoperiodic type. A classic example of this phenomenon is the cultivated strawberry (*Fragaria* × *ananassa* Duch.) (Fig. 8.4). In strawberries, all three photoperiodic types are represented in standard commercial cultivars. Traditional 'Junebearing' cultivars are short-day plants, 'Everbearers' are long-day plants and 'Day-neutral' are day-neutral plants. Day-neutral cultivars are often called everbearers, however, the two are distinct cultivar types. The physiology of strawberry flowering is greatly influenced by temperature. Junebearers are most sensitive to temperature, everbearers are moderately sensitive to temperature and day-neutrals are relatively insensitive to temperature with respect to flowering.

Fig. 8.4. A strawberry (*Fragaria* × *ananassa*) plant with stolons (runners) and fruit. Both flowering and stolon production are true quantitative photoperiodic developmental responses to light.

In all photoperiodically sensitive crops there are two processes at work which result in the manifestation of the photoperiodic response. One is the circadian rhythm, regulated by cryptochromes and the other is the photoperiodic response regulated by phytochrome. The circadian rhythm maintains a 24 h cycle of light sensitivity, which must be considered in any scheme where photoperiod is to be manipulated for horticultural production. Timing of light, dark, and NI cycles is crucial for success. Phytochrome controls the sensitivity to light and dark within the 24 h. There is a periodicity to the wavelength sensitivity of many species. In other words, the plant will only respond to specific wavelengths (i.e. red or far-red) if given at the appropriate time during the 24 h cycle.

Plants can be qualitative or quantitative in their photoperiodic requirement and also absolute or facultative. In addition, there can be combinations of the two. Quantitatively photoperiodic means

that the intensity of flowering is directly related to the daylength treatment imposed on the plant. Qualitatively photoperiodic means that the intensity of flowering is not directly related to the photoperiod treatment. Absolute means that there is an absolute requirement for a specific number of photoperiodic cycles at a particular daylength for flowering. Facultative means that the photoperiodic requirement is not an absolute requirement, but rather that flowering is greatly stimulated with the prescribed treatment.

Putting this all together, plants are usually one of the following with respect to flowering: (i) absolute quantitative short-day plants; (ii) absolute qualitative short-day plants; (iii) facultative quantitative short-day plants; (iv) facultative qualitative short-day plants; (v) absolute quantitative long-day plants; (vi) absolute qualitative long-day plants; (vii) facultative quantitative long-day plants; (viii) facultative qualitative long-day plants; and (ix) day-neutral plants. Most discussions of photoperiodic type with respect to flowering are limited to long-day, short-day, or day-neutral.

Some common long-day plants include *Rudbeckia*, California poppy (*Eschscholzia californica*), radish (*Raphanus sativus*), lettuce (*L. sativa*), and spinach (*Spinacia oleracea*). Short-day plants include chrysanthemum (*Chrysanthemum* spp.), poinsettia (*Euphorbia pulcherrima*), and Christmas cactus (*Schlumbergera* spp.). Day-neutral crops include tomato (*Solanum lycopersicum*), corn (*Zea mays*), and cucumber (*Cucumis sativus*).

Besides flowering, some other responses to photoperiod that are horticulturally important include, but are not limited to, tuber formation in potato (*Solanum tuberosum* L.), bulb formation in onion (*Allium cepa*) and garlic (*Allium sativum*), and runner or stolon formation in strawberries (*Fragaria* × *ananassa*).

Circadian Movements and Rhythms

Rhythmic growth patterns in plants often follow a general 24 h cycle. These rhythmic patterns are part of the circadian rhythm present in nearly all living organisms. When a particular growth movement is truly part of the circadian rhythm, it remains even if the plant is transferred to constant darkness for several 24 h cycles in a row. A great example of such a growth pattern occurs with leaves of the common bean (*Phaseolus coccineus*) which rise and fall following a rhythmic cycle. This movement is temperature independent. Opening and closing of flowers of some species are highly regulated by the time of day, for example in morning glory (*Ipomea* spp.) and angel's trumpet (*Brugmansia* spp.).

This rhythmicity is controlled by two factors: (i) genetics; and (ii) some factor in the environment which triggers the start of the cycle. This trigger is normally light in the blue or UV-A range and is perceived by cryptochrome. The genetic component is an autonomous regulation of growth that is not affected by external stimuli. Phytochrome may also be involved in setting the length of the circadian rhythm. Far-red light tends to shorten the length of the periods and also induces a quick subsiding of the rhythm.

Photosynthesis, Plant Growth, and Yield

So far in this chapter we have looked at how light regulates growth and development utilizing the photoreceptors phototropin, cryptochrome, and phytochrome. Light is also important as it drives one of the most important biochemical processes on earth, photosynthesis. Rather than study photosynthesis from a purely biochemical basis, we will approach it from a horticultural perspective by investigating aspects of plant biology that influence photosynthesis and ultimately, plant productivity.

A quick general review of photosynthesis

Photosynthesis, simply put, is the transformation by organisms in the *Plantae* kingdom of light energy into stored chemical energy. This stored energy can now be utilized by the storing organism or by organisms that do not possess the ability to directly use light as a source of energy. This includes human beings. With few exceptions, all living organisms on earth rely on photosynthesis for survival.

Though this is a simple description of photosynthesis, it is not a simple process. Photosynthesis is a very complex process requiring specific physiological and anatomical characteristics for completion. The physiological processes of photosynthesis are usually divided into two major categories: (i) the light reactions; and (ii) the dark reactions. The light reactions only occur in the light and result in the formation of high energy compounds (temporarily storing light energy from the sun) which are utilized in the dark reactions. In addition, oxygen is released from water molecules, thereby providing

the oxygen needed for aerobic respiration. Some of this oxygen is converted to ozone in the upper part of the earth's atmosphere, protecting the earth from UV radiation. The light reactions take place on the thylakoid membrane of the chloroplast. The dark reactions can occur in the light or the dark and take the stored energy from the light reactions and transfer it to biologically stable storage compounds. The dark reactions take place in the liquid part of the chloroplast called the stroma.

The light reactions

In order to accomplish these tasks, most photosynthesizing organisms of horticultural importance have very specialized organs (leaves) containing the principal components of the photosynthetic machinery. The first step in photosynthesis is capturing light energy. This is accomplished by specialized pigments which absorb light in the 400–700 nm range. Recall this part of the spectrum is called PAR. The two major pigments that absorb light energy for photosynthesis are the chlorophylls and the carotenoids. Chlorophyll molecules are anchored to the thylakoid membrane of the chloroplast. The chlorophyll molecule contains a magnesium atom, hence its importance in soil fertility discussions. Chlorophyll absorbs blue and red light while reflecting or transmitting green light.

Carotenoids are yellow-orange accessory pigments that absorb light in the blue portion of the spectrum, where chlorophyll does not, to extend the range of the light-spectrum energy participating in photosynthesis. Accessory pigments are those pigments other than chlorophyll that participate in photosynthesis. Carotenoids also help protect chlorophyll molecules from ROS.

When a photon of light at the appropriate wavelength hits a pigment molecule, an electron in the pigment molecule absorbs the energy and becomes 'excited'. The energy of this excited molecule can be passed from one molecule to another in a carefully orchestrated manner in the thylakoid, ultimately being passed to a specialized structure in the thylakoid called a reaction center. There are two types of reaction centers: (i) photosystem I (PSI or P700); and (ii) photosystem II (PSII or P680). These reaction centers collect and concentrate the energy absorbed by pigment molecules. Energy is passed from pigments to reaction centers. In addition, energy from PSII is passed to PSI. When a molecule of chlorophyll in the reaction center is excited, it passes this energy along a series of carrier molecules. As the energy is passed along, it is used to synthesize adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH). The ATP and NADPH now store the energy from the sun until it can be used to reduce CO_2 to form sugars, where the energy can be stored in a biologically stable form for a longer time.

In this process of energy movement within the thylakoid from pigment to reaction center to either ATP or NADPH, electrons are removed from PSII. These electrons must be replaced in order for the process to continue. Water molecules are split, releasing oxygen, which diffuses out of the chloroplast and eventually exits the leaf through open stomata. The H^+ replace the electrons removed from PSII.

The dark reactions

At this point we have light energy from the sun temporarily stored in ATP and NADPH. The processes in photosynthesis that transfer this energy from ATP or NADPH to CO_2, reducing the CO_2 to a carbohydrate are called the dark reactions. Remember, the dark reactions can take place in either the light or the dark but they require the products of the light reaction (ATP and NADPH) to proceed. This conversion of CO_2 to carbohydrate is accomplished in a series of biochemical reactions called the Benson-Calvin cycle, the Calvin cycle or the reductive pentose phosphate pathway. These reactions take place in the stroma of the chloroplast.

This reduction of CO_2 to carbohydrate takes many steps, each of which is accomplished by a specific enzyme. CO_2 combines with a five carbon compound called ribulose-1,5-bisphosphate (RuBP) to form a six carbon molecule which quickly splits into two three carbon molecules of 3-phosphoglycerate. Energy from ATP or $NADPH_2$ convert the molecules of 3-phosphoglycerate into two molecules of glyceraldehyde 3-phosphate (G3P). For every three molecules of CO_2 incorporated into the Calvin cycle, one molecule of G3P is produced to be converted into a sugar for storage, or another compound needed for general metabolism. For each G3P synthesized, the cycle spends six molecules of ATP and six molecules of $NADPH_2$. The remaining G3P are used along with three more ATP to regenerate RuBP so that it can fix more CO_2. The enzyme which catalyzes the first step of the Calvin cycle is RuBP carboxylase or RuBisCo and it is the most abundant protein on earth.

With a basic knowledge of the processes occurring in photosynthesis, we can now look at a vast array of factors that affect photosynthesis and ultimately horticultural productivity.

Factors affecting photosynthesis

Leaves are the primary photosynthetic organ of a plant. Their unique structure, shape, position, and biochemistry provide them with the opportunity to absorb a maximum amount of solar radiation for manufacturing growth and storage compounds. Just how much of this solar energy is converted into biologically useable energy depends on many factors, some of which are environmental others are plant based.

Plant-based factors affecting photosynthesis

LEAF STRUCTURE, BIOCHEMISTRY, AND PHOTOSYNTHESIS Photosynthesis occurs primarily in the leaf lamina or blade. Stomata orchestrate gas exchange between the interior of the leaf and the environment and their position and number vary widely among species. In addition, the epidermis of a leaf may include a waxy cutin and/or hairs to minimize water loss. Leaf position may change via specific plant movements to maximize light interception.

Inside, leaf anatomy is an amazing array of palisade cells near the adaxial (upper) surface vertically oriented to maximize light interception subtended by a collection of spongy mesophyll cells with abundant air spaces for gas exchange. Within the spongy mesophyll are intricate networks of vascular tissue to bring water to the leaf and shuttle products of photosynthesis off to awaiting sinks.

At the cellular level, leaves are equally amazing. Within mesophyll cells are the powerhouses of photosynthesis, chloroplasts, each cell containing from 20 to 100 of them. Chloroplasts can change their position within the cell, adjusting to changing light conditions. Each individual chloroplast increases the photosynthetic area of the leaf.

Plants have four main biochemical/anatomical avenues for fixing CO_2: (i) C3; (ii) C4; (iii) C3-C4 intemediates; and (iv) crassulacean acid metabolism (CAM) photosynthesis.

Most monocots and dicots of the temperate zone have C3 photosynthesis. It is called C3 photosynthesis because the first stable product of carbon fixation is a three carbon molecule. C3 plants generally have a low water use efficiency and waste much of the energy they fix in a process called photorespiration. Photorespiration is the process where the enzyme normally fixing CO_2 instead fixes O_2 wasting much of the energy harvested in the light reactions and releasing CO_2, rather than fixing it. This is important: not only is O_2 taking the place of CO_2 in reactions with the RuBisCo enzyme, in the process it is releasing CO_2 previously fixed. As much as 30–50% of the energy stored in ATP and NADPH in the light reactions can be wasted in photorespiration. Photorespiration occurs when the concentration of CO_2 falls to 50 ppm or lower. This can occur under hot, dry conditions when stomata close to conserve water.

In photorespiration, instead of forming two three carbon G3P molecules like it does when RuBisCo fixes CO_2, only one molecule of G3P is produced and a toxic two carbon molecule called phosphoglycolate is produced. Since it is toxic, the plant must get rid of the phosphoglycolate. It does so through a series of reactions involving peroxisomes and mitochondria. First the phosphoglycolate is converted to glycolic acid, transported to the peroxisome and converted into glycine. The glycine is then transported to a mitochondiria and converted into serine and then glycerate. The glycerate is shuttled back to the chloroplast, phosphorylated and re-enters the Calvin cycle. In this process, substantial metabolic energy is used and CO_2 is lost from the plant.

Most grasses and cereals common to the tropics have C4 photosynthesis. They are generally much more efficient than C3 species because they have an anatomical/biochemical mechanism for maintaining high CO_2 levels around the RuBisCo enzyme which is only found in specialized cells surrounding vascular tissues. These specialized cells are called bundle sheath cells and this arrangement of leaf cells is called Kranz anatomy. Since RuBisCo is not present in mesophyll cells, photorespiration cannot occur. The first stable products of carbon fixation which occurs in mesophyll cells is a four carbon compound, oxaloacetate, thus they are called C4 plants. The oxaloacetate is converted into malate or aspartate, both four carbon compounds, which are then shuttled to bundle sheath cells where CO_2 is released from the four carbon compound and fixed by RuBisCo in the Calvin cycle. C4 plants use water more efficiently and are generally more productive than C3 plants.

Some species (*Flaveria, Panicum*) have Kranz anatomy but lack the C4 biochemistry. These species

are called C3-C4 intermediates. Their photosynthetic and photorespiratory pathways are identical to C3 species, and RuBisCo is found in both mesophyll and bundle sheath cells. Any CO_2 released during photorespiration is refixed via an enzyme called glycine carboxylase of bundle sheath mitochondria. Species in the C3-C4 intermediate group use water more efficiently than C3 species and are therefore more productive than C3 species.

CAM species have the biochemistry of C4 species and the anatomy of C3 species. Carbon fixation is separated in time, rather than anatomically as in C4 plants. Mespophyll cells of CAM plants do not differentiate into palisade and spongy layers, but rather, they are all spongy. They have fewer stomata than C3 or C4 plants and the stomata stay open at night to fix CO_2 to be stored as malate in the vacuole until the following day. During the day, stomata are closed to minimize water loss, malate from the vacuole is converted into phosphoenolpyruvate and CO_2 is released to be fixed by RuBisCo in the Calvin cycle. CAM plants are generally inefficient since they exhibit photorespiration and they do not use water very efficiently.

LEAF POSITION Leaf exposure to solar radiation can be greatly affected by the leaf's position in the plant canopy. Leaves at the top or exterior of the canopy generally have high levels of electron transport in the light reactions of photosynthesis and concomitantly high rates of RuBisCo activity and carbon assimilation. Leaves lower or more interior in the canopy generally have much reduced light reactions and subsequently, reduced carbon fixation in the dark reactions.

LEAF AGE Photosynthetic rates steadily decline with leaf age. In fact, leaf age is more of a factor in photosynthetic rates than light intensity. Younger leaves may not be light saturated at 1800 μmol/cm^2/s while older leaves might be nearly 90% saturated at 600 μmol/cm^2/s. Light saturation of photosynthesis occurs when increasing the light intensity will not increase the rate of photosynthesis. Some other factor of photosynthesis is limiting photosynthetic rate, in this case, leaf age.

In addition, older leaves of C4 species may exhibit photorespiration due to a diminished capacity to concentrate CO_2 in the bundle sheath cells. When chlorophyll and RuBisCo content of senescing C4 leaves reaches 50% of normal mature leaves, photorespiration can approach that in C3 plants.

Environmental factors influencing photosynthesis

Plants are immobile and as such are subjected to a wide array of environmental conditions which may enhance or hinder photosynthesis. In this section we will look at environmental factors that may have either a direct or an indirect effect on photosynthesis and plant productivity. These factors include: (i) CO_2 levels; (ii) temperature; (iii) light (and shading of leaves); (iv) water stress (drought, excess water and anaerobiosis); (v) soil salinity; (vi) gaseous pollutants; and (vii) heavy metal contamination of soil.

CO_2 CONCENTRATION

DEFICIENCY CO_2 is the basic substrate of photosynthesis. Regardless of species, low CO_2 levels lead to lower rates of photosynthesis. Some species are just much better at utilizing lower levels of CO_2 than others.

ELEVATED In general, increased CO_2 leads to increased growth and productivity of most species. The levels of RuBisCo ultimately determine the amount of photosynthesis that can occur. In general, increasing CO_2 concentration does not induce a change in RuBisCo content of leaves. The effect of CO_2 concentration on the activity of RuBisCo in leaves is not clear, with some studies indicating an increase, some a decrease, and still others no change with increased CO_2.

Increases in yield observed with elevated CO_2 may be a reflection of physical changes in leaves that often occur with elevated CO_2. These changes include an increase in leaf area and fresh weight, thicker leaves, and a greater number of palisade cells.

TEMPERATURE

HEAT An increase of 10–15°C above the normal growing temperatures (15–45°C) for a crop generally leads to a reduction in photosynthesis. In general, photosynthesis inhibition at higher temperatures is greater in C3 plants compared with C4 and CAM plants. The reduction in photosynthesis is caused by a myriad of factors including: (i) disruption of thylakoid membranes; (ii) reduction in electron transport chain activity; (iii) disruption of PSII; (iv) grana unstacking; (v) reduced O_2 evolution from photosynthesis; and (vi) denaturation and inactivation of many enzymes, especially RuBisCo and to a lesser degree phosphoenolpyruvate carboxylase (PEPCase). In addition, photosynthetic

rate is reduced more than respiration rate during heat stress leading to a general loss of carbon during heat stress.

CHILLING Chilling (exposure to temperatures between 0 and 15°C) inhibits photosynthesis particularly in tropical and subtropical species. Temperate species may also suffer chilling injury, however, the temperature range defining chilling is usually reduced to 0–5°C. The extent of the injury depends on: (i) temperature; (ii) length of exposure; (iii) developmental stage of the crop; and (iv) species and cultivar.

Inhibited photosynthesis results from changes in membrane fluidity, reduced enzyme activity, slower protoplasmic streaming, chloroplast swelling, inhibition of PSI and PSII, and increased susceptibility to photoinhibition at low temperatures. High light levels during chilling damages PSII and photoinhibition readily occurs. Photoinhibition may also occur under low light conditions at chilling temperatures. One of the major reasons for photoinhibition at chilling temperatures is a decrease in the activity of oxygen-scavenging enzymes.

In many crop species, photosynthesis at 10°C is much lower than at 20°C. Chilling injury begins to occur when membranes acquire more saturated fatty acids in direct response to exposure to low temperatures. Increased saturated fatty acid content makes membranes less fluid leading to disrupted light harvesting, electron transport and enzyme activity. RuBisCo and to a much lesser extent PEPCase activity is inhibited by chilling temperatures. Chilling causes an accumulation of starch and sucrose and sucrose may play a bioprotective role during chilling.

LIGHT Light drives photosynthesis. Increasing light levels will increase photosynthetic rates accordingly but only up to a point. When all other factors are not limiting and increasing light levels does not increase photosynthesis rates, we have reached the *light saturation point*. Many C3 species are saturated at low light levels due to photorespiration while most C4 species are never light saturated. Reducing light levels of leaves at or below the saturation point results in decreased photosynthesis. These responses to light are instantaneous. Long-term changes in light level can also influence photosynthesis. Under reduced light levels, photosynthesis is reduced due to lower stomatal conductance and reduced mesophyll area.

Shading of leaves can greatly influence their photosynthetic potential. Shade leaves generally have a lower light saturation point compared with similar leaves grown in the sun. Thus even if these leaves were to become well lit their photosynthetic rate would still be compromised. Shading itself causes a reduction in RuBisCo activity and electron transport which leads to reduced photosynthesis.

WATER STRESS

DROUGHT One of the first plant responses to water stress is stomatal closure. When a leaf becomes dehydrated due to water stress, abscisic acid (ABA) transported from roots as well as ABA formed in mesophyll cells induces stomatal closure. Stomatal closure leads to decreased internal leaf CO_2 content and a concomitant reduction of photosynthesis.

Lack of sufficient water leads to a decrease in leaf water content which causes cells to shrink. Solutes become more concentrated and the plasma membrane becomes compressed. These conditions lead to decreased cell expansion and decreased leaf size. Over time, fewer and smaller leaves are produced leading to an overall reduction in leaf area per plant (i.e. a reduction in LAI). Photosynthesis on a whole-plant basis is thereby reduced.

Permanent scars occur on membranes of the grana due to water stress to chloroplasts. In addition, many thylakoid proteins are damaged by oxidation and deleterious structural changes occur in the chlorophyll–protein complexes of the thylakoid. PSII photochemistry is only slightly altered by drought stress, even though efficiency of PSII may be reduced by drought stress. The reduced efficiency is brought on by aberrations of the electron transport system and photoinhibition rather than a direct effect on PSII. Chloroplasts of bundle sheath cells seem to be more resistant to drought stress than those of mesophyll cells.

Water stress also leads to reduced photophosphorylation and decreased ATP synthesis caused by a reduction in the synthesis of the ATP synthase which is brought on by an increase in magnesium concentration in the chloroplast. Reduced ATP content limits RuBP biosynthesis which leads to reduced photosynthesis.

Reduced photosynthesis under drought stress results from inhibited regeneration of RuBP and reduced levels and activity of RuBisCo. C4 plants require less RuBisCo than C3 plants for a similar rate of photosynthesis, thus C4 plants are less sensitive to drought stress than C3 plants.

However, PEPCase is inhibited under water stress, and ultimately drought stress reduces C4 photosynthesis too. Dehydration of chloroplasts under water stress may lead to conformational changes in these enzymes, further limiting their activity. Sulfate and phosphate anions accumulate in the dehydrated chloroplasts and can directly inhibit RuBisCo activity. Chloroplasts also become more acidic under water stress which might reduce RuBisCo activity.

Water stress often leads to elevated sucrose and reduced starch levels in leaves largely due to a remobilization of starch into sucrose under carbon limiting conditions. The activity of the two enzymes important in sucrose biosynthesis, cytosolic fructose-1,6-bisphosphatase (FBPase) and sucrose phosphate synthase, declines in water-stressed leaves. Production of assimilates is reduced by water stress, but their translocation is relatively unaffected.

EXCESS WATER AND ANAEROBIOSIS Poor soil drainage or excessive rainfall or irrigation leads to anaerobic soil conditions. Plants grown under anaerobic conditions exhibit reduced photosynthesis, slow growth, and drastically reduced yield. A shortage of oxygen in the roots is believed to stimulate ABA production in the roots which is then translocated to leaves causing stomatal closure. With closed stomata, CO_2 diffusion into the leaves is severely restricted creating a lack of substrate for photosynthesis. In addition, anaerobiosis accelerates carbohydrate breakdown, further reducing the already decreased carbon reserves within the plant.

SOIL SALINITY Salts, particularly chlorides and sulfates of sodium, magnesium, and calcium, often accumulate in the soil due to irrigation with poor quality water. This problem is often exacerbated by poor drainage and the lack of periodic flushing of salt from irrigated soils and draining away of the flushing water. Sodium chloride is a particular problem due to its very high solubility.

Excessive soil salinity induces osmotic stress, direct ion toxicity, and ionic imbalance in plants. Osmotic stress results from the increasingly negative osmotic potential of the soil solution with increasing salinity. Direct ion toxicity from sodium, chloride or sulfate ions taken up from the soil solution also occurs. Nutrient imbalances develop as unwanted ions compete with desirable nutrient ions for uptake by the roots. All three products of saline soil have deleterious effects on photosynthesis, particularly in arid and semi-arid regions where soil salinity is a particular problem.

Plants under salt stress conditions exhibit a decrease in photosynthesis primarily due to a decrease in stomatal conductance due to stomatal closure as a result of osmotic stress, sodium toxicity, and elevated ABA content of guard cells induced by osmotic stress in the roots. When stomata close, the internal CO_2 concentration declines and photosynthesis is reduced. The initial response to salt stress is the osmotically induced closing of stomata, followed after further salt exposure by ion toxicity responses.

Species vary widely in their tolerance of saline soil. Spinach (*Spinacia oleracea*) can tolerate high levels of salt toxicities with no decrease in photosynthesis even though stomatal conductance and internal CO_2 concentrations are reduced under the stress. On the other hand, rice (*Oryza sativa* L.) exhibits a marked reduction in photosynthesis with a decrease in stomatal conductance under saline conditions.

Reduced photosynthesis under saline conditions can also be attributed to alterations in chloroplast structure. Salinity induces an accumulation of sodium and chloride ions within chloroplasts leading to shrinking of the thylakoid membrane and stacking of adjacent membranes in the grana. Chlorophyll a molecules are destroyed by saline conditions thus total chlorophyll levels decline. In addition, chlorophyll tends to become loosened from its protein. Salt-tolerant species tend to avoid degradation of chlorophyll molecules under saline conditions by sequestering sodium in vacuoles and producing osmolytes such as putrescine and quaternary ammonium compounds in the chloroplasts.

Salinity increases a plant's susceptibility to photoinhibition which leads to the production of toxic singlet oxygen in chloroplasts and degradation of PSII under excessive light conditions. With a compromised PSII, photosynthesis is greatly inhibited. Electron transport is inhibited by salinity in some species but not in others.

The level and activity of RuBisCo decreases under saline conditions. The activity of PEPCase, which is important in C4 photosynthesis and a host of other plant processes including C/N partitioning in C3 leaves, guard cell carbon metabolism, seed formation and germination, and fruit ripening, rises considerably under salt stress.

Photosynthesis produces primarily sucrose and starch. Salinity induces an accumulation of starch and sucrose attributable to impaired carbohydrate in respiration. In addition, sucrose may serve an osmoprotectant role under salt stress conditions. Accumulation of photosynthetic products tends to inhibit photosynthesis.

GASEOUS POLLUTANTS When wood, coal or petrochemicals are burned for fuel, gases such as CO_2, CO, SO_2, NO, NO_2, H_2S, HF, and ethylene are released into the atmosphere. In high concentrations these pollutants reduce photosynthesis and inhibit plant growth.

Increased CO_2 in the atmosphere leads to increased absorbance of infrared light resulting in global warming. In addition, high levels of CO_2 may cause stomatal closure, resulting in decreased photosynthesis.

SO_2 enters leaves through stomata and dissolves in the cytoplasm forming bisulfite and toxic sulfite ions. NO and NO_2 are also absorbed by leaves through stomata and may directly inhibit photosynthesis.

One of the most phytotoxic agents produced through a reaction of sunlight with air containing hydrocarbons and nitrogen oxides is ozone. Ozone levels are most often high during the summer months near urban areas. Ozone is a very potent oxidizing agent, leading to a number of physiological problems. Ozone inhibits translocation of photosynthates by interfering with phloem loading. This leads to excessive starch accumulation, reduced photosynthesis, destruction of the chlorophyll molecule followed by bleaching of photosynthetic pigments. Ozone also damages the chloroplast envelope and thylakoid membranes directly, leading to further reduction in photosynthesis. The level and activity of RuBisCo decreases upon exposure to elevated ozone levels and photoinhibition is promoted even at moderate light levels. Ozone also interferes with efficient guard cell regulation of stomatal opening.

Degradation of ozone in the cytoplasm leads to the production of ROS such as the superoxide anion, singlet oxygen, and hydroxyl radicals. These ROS lead to damaged proteins and nucleic acids along with peroxidation of lipids.

Beneficial ozone, located high in our stratosphere, forms through a reaction of oxygen with UV light. Once present, the ozone layer helps filter out UV rays that would be harmful to life on earth. Humans must not return to activities which result in a depletion of the stratotospheric ozone like that which occurred with the use of chlorofluorocarbons as aerosol spray propellants. Depletion of this ozone layer allows UV-B light to reach the earth's surface in ever increasing amounts. UV-B light causes stomatal closure, reduced RuBisCo production, and it directly damages the photosynthetic machinery reducing photosynthesis in C3 and C4 plants. Reduced photosynthesis leads to not only reduced photosynthates but reduced oxygen production as well.

HEAVY METAL CONTAMINATION OF SOIL Heavy metals such as cadmium, nickel, mercury, copper, zinc, lead, and aluminum have serious negative consequences for plant productivity. The presence of one or many of these metals in the soil may lead to reduced chlorophyll content and disorganized or destroyed chloroplast structure which leads to reduced photosynthesis. Most of these metals interfere with PSII. Cadmium and lead inhibit chlorophyll and carotenoid synthesis and alters the ultrastructure of chloroplasts. Excess copper inhibits the electron transport between PSII and PSI and significantly reduces oxygen evolution from PSII. Mercury and nickel inhibit both photosystems. Manganese toxicity reduces photosynthesis through peroxidative damage to the thylakoid membrane. Cadmium and nickel lead to reduced carbon fixation through direct effects on Calvin cycle enzymes. Many heavy metals also induce the formation of free radicals leading to severe oxidative damage.

Plant productivity and photosynthesis

Our interest in photosynthesis is linked to our interest in maximizing horticultural productivity. In this section we will examine many of the components that determine and regulate productivity of horticultural crops and how they relate to photosynthesis. A good place to start our investigation is to look at the whole plant, its canopy, and subsequent crop productivity.

THE CROP CANOPY AND PRODUCTIVITY A crops canopy consists of all above-ground organs which intercept sunlight including leaves, flowers, fruit, and the stem which supports them all. Of these components of the canopy, we are mostly interested in leaves and how they affect productivity and yield.

LEAF AREA INDEX (LAI) A common measure discussed in relation to productivity is the LAI.

This is a measure of the amount of leaf area covering a unit area of ground. Crop productivity does not rely on leaf size or leaf area per plant, but rather, on this measure of leaf area per unit land. LAI is usually expressed as square meters (m^2) of leaf area per square meter (m^2) ground area. LAI is important as a measure of the potential leaf area for intercepting solar radiation per unit land area.

It is also important to know not only how much light hits the canopy, but what happens to the light of different wavelengths once it hits and penetrates the canopy. PAR (400–700 nm) hitting the canopy is either reflected (15%), absorbed (75%), or transmitted (10%). UV radiation (<380 nm) and long wave (>4000 nm) are both nearly 100% absorbed. Less than 25% of near infrared radiation (750–1200 nm) is absorbed. The rest is reflected and transmitted.

While leaf absorption characteristics are important in understanding what happens inside a canopy, how much radiation penetrates the canopy and how far it gets is the most important factor to consider with yield. A formula for calculating the solar penetration into a canopy is:

$$I = I_0 e^{-kL}$$

Where:
I = solar penetration into a canopy (the approximate percentage of the solar radiation hitting the top of the canopy that reaches the height determined by the calculation of L)
I_0 = the solar radiation at the top of the canopy in W/m^2
e = the base of the natural logarithm (approximately equal to 2.71828)
k = the extinction coefficient for a particular crop canopy
L = the cumulative LAI from the top of the canopy down to the selected height
The extinction coefficient is a measure of the amount of light lost to scattering and absorption as light passes through a medium, in this case, the plant canopy. The extinction coefficients range from 0.57 for fescue (*Festuca arundinacea*), 0.59 sugarcane (*Saccharum officinarum*), 0.76 for clover (*Trifolium repens*), 0.84 for rape (*Brassica napus*), and 0.88 for alfalfa (*Medicago sativa*). Grasses or other crops with somewhat vertically oriented leaves have extinction coefficients less than 0.6 while broadleaf species or those with more horizontally positioned leaves may have extinction coefficients over 0.7. More horizontally oriented leaf blades absorb more light, thus less of the incident light travels through to leaves below.

As an example, if the extinction coefficient is 0.5, then the solar radiation reaching the bottom of a canopy with a LAI of 3 m^2/m^2 is 22% of the light level hitting the top of the canopy. If the LAI is higher, say 5 m^2/m^2, then that level drops to 8.5%. Higher LAIs result in reduced light penetration into the canopy. While it is good to have a high LAI for productivity, LAIs that are too high can be detrimental to productivity.

Some of the incident radiation is re-reflected, generally in the range from 15 to 25%. Vertically oriented narrow leaves reflect less while horizontal leaves reflect more radiation. Sun angle is also a factor. When the sun is closer to the horizon, reflection increases.

When considering LAI, it is important to understand that it changes during the growing season. With annual crops and deciduous perennials, LAI starts out at or near 0 m^2/m^2 in the spring. Depending on growth habit and planting density, this value steadily increases to its maximum then begins to decline as senescence sets in. When the LAI reaches about 3 m^2/m^2, nearly all solar radiation is absorbed by the leaves. In most crops, maintenance of a LAI near 3 m^2/m^2 is desired for maximum production. Even though some leaves are lost during the growing season to pests or natural senescence, newly emerging leaves generally replace the lost ones. While leaf senescence is undesirable for much of a plant's growth, crops which store large amounts of protein often rely on leaf senescence for the translocation of the protein from the leaf to the storage organ.

SOLAR ENERGY AND PRODUCTIVITY A high LAI does not necessarily lead to high yield. Leaves must use the solar radiation they receive efficiently and convert that energy into usable biological energy. The efficiency of solar radiation use by leaves has not really changed over the last 100 years, even though yields have increased dramatically. The yield increases observed are mostly due to increases in dry matter allocation within crop plants to the harvested portion of the plant. This is often measured in harvest index (HI) which represents the yield of harvested commodity per unit total plant biomass, both usually expressed as dry weight. In a very general sense, the HI has increased from about 0.3 to 0.6 in the last 100 years. Further increase in the HI is limited since a certain portion of a plant's

biomass is required for activities other than existing as the harvested commodity. In other words, a wheat plant cannot produce only wheat kernels; it must also have roots, stems, and leaves to produce the grain. Further enhancements to yield will probably come from increases in solar use efficiency which would allow higher plant densities without a concomitant reduction in production per plant.

Solar energy efficiency of leaves considers that for every 1 mol of CO_2 fixed into plant biomass, 8–10 mol of PAR light quanta are needed. This is approximately a 20% conversion ratio or energy efficiency. The global solar efficiency looking at total incoming radiation and total global biomass produced is around 0.1% total or 0.2% PAR. Most crops have solar efficiencies of about 0.5–3%. This corresponds to about 1–3 g dry matter/MJ PAR received. Theoretical estimates of solar efficiencies suggest that it could be increased to 4–6 g dry matter/MJ PAR. Such an increase would probably be achieved through genetics and to a lesser degree by modified production practices.

Assimilate production efficiency is higher under lower light levels and decreases as light levels increase. More efficient use of incident radiation comes from a well-designed canopy, as in many pruned and trained orchards and vineyards. The total light hitting the plant is more evenly distributed among all the leaves. In order to achieve this optimum light distribution among leaves, a much larger LAI is needed.

To more fully understand this idea, consider several different canopy arrangements. In a canopy where leaf orientation is at 90°, all leaves would intercept direct radiation, their rate of photosynthesis would be high, but their solar use efficiency would be relatively low because of the high light irradiance. If leaves in another canopy were oriented at a 20° from vertical their rate of photosynthesis would be low, but their solar efficiency would be relatively high, due to the lower irradiance.

In an ideal situation, leaves should be neither vertically nor horizontally oriented, but rather, be oriented at different angles at different levels of the canopy (Fig. 8.5). Leaves high in the canopy should be oriented more vertically while a more horizontal component should occur as one descends the canopy height. This arrangement produces moderate rates of photosynthesis with moderate efficiencies in all leaves of the canopy.

The most desirable situation for an annual crop would be one where the first leaves to emerge would be primarily horizontally oriented to cover the ground area quickly. As the plants grew, leaves would take on a more vertical orientation to achieve the desired distribution. While production methods for the most part cannot initiate such growth, plant breeders can select for plants exhibiting this trait or any tendency towards it. Many modern cereal cultivars have nearly vertical leaves, allowing LAIs of close to 6 m^2/m^2. Nearly all leaves, sheaths, and internodes contribute to assimilate production, since they are all moderately irradiated. Cereal crops also have the advantage of producing tillers for filling in space rapidly leading to high LAIs as compared with higher density seeding which would cost more to implement.

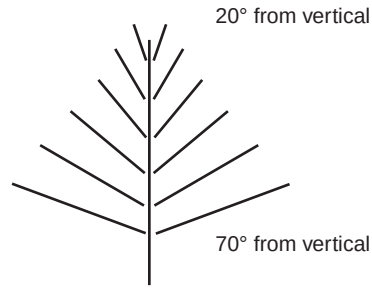


Fig. 8.5. Leaf orientation should transition from more vertical higher in the canopy to more horizontal lower in the canopy for optimum productivity.

Excessively high LAIs are not desirable since on days of low irradiance, lower leaves would respire more carbon than they fix, resulting in a net carbon loss.

SOURCES, SINKS, AND PHOTOSYNTHESIS The first product of carbon fixation in the leaf, triose phosphate, can be converted into starch in the chloroplast or shuttled to the cytoplasm to form sucrose. The formation, translocation, and storage of carbohydrates is an important topic of horticultural physiology since we rely on many crops for sucrose and starch for food or feed. The partitioning of carbohydrate into starch (immobile) or sucrose (mobile) occurs not only at the cellular level, but at the whole plant level as well. Organs of active photosynthesis, mainly leaves but sometimes stems and fruit, are called 'sources' while recipients of translocated photosynthates are called 'sinks'. How efficient a plant is with respect to carbon partitioning among sources and sinks often determines crop productivity.

Metabolism of starch in the chloroplast is dynamic. Up to 30% of the CO_2 fixed by a plant is incorporated into starch during the day and accumulates as granules in the chloroplast. The cytoplasm is not involved in starch synthesis. At night, starch is decomposed in the chloroplast into triose phosphates and exported to the cytoplasm where sucrose is synthesized and loaded into the phloem for translocation to other plant tissues (sinks) for metabolism or conversion back into starch for storage.

The carbon fixed in photosynthesis must be allocated among:

- sucrose for translocation;
- starch for storage; and
- carbohydrates for metabolism.

Some of the factors that regulate this process include: (i) rate of assimilate translocation; (ii) respiratory needs; (iii) use of carbon in other biosynthetic pathways; (iv) variations in photosynthetic rates; and (v) nutrient availability to the plant which regulates general plant growth and carbon needs. Changing growth rates in different parts of the plants cause fluctuating demands for skeletal carbon and energy. Different stages of tissue growth also cause differences in both assimilate supply and demand. Starch formation is highly dependent on photosynthesis: high rates of photosynthesis translate into high rates of starch formation. On the other hand, sucrose synthesis is somewhat independent of photosynthetic rates. Sucrose synthesis is fairly constant throughout the diurnal cycle. The source of substrates change from newly fixed triose phosphates in the light to those derived from degraded starch in the dark.

Both starch and sucrose syntheses are subjected to endogenous rhythms in plants. When plants are moved from normal growing conditions to continuous lighting, the accumulation of starch shows a remarkable decrease as the plant enters the time of day near the end of its normal light period, even though it was under continuous lighting. In addition the photosynthetic rate slowly declines as a plant enters the time of its normal dark period even though it was still illuminated.

Part of the photoassimilated carbon remains in the photosynthetic tissue and is utilized to satisfy the many biosynthetic reactions occurring within the leaf cell. In particular, much of the carbon flows into the respiratory metabolism. Respiration plays an important function in green cells, even under light conditions. For instance, routing carbon to the tricarboxylic acid cycle provides carbon skeletons for amino acid biosynthesis.

The general mechanism for sucrose movement from the source to the sink is the pressure flow or mass flow mechanism. Sucrose can move from the mesophyll cells to the phloem either symplastically through plasmodesmata or apoplastically via active phloem loading into sieve cells or through a combination of the two routes. There is extensive evidence supporting a mostly apoplastic mechanism for phloem loading via an ATP-driven transporter which concomitantly pumps H^+ into the apoplast and sucrose into the sieve cell.

Sucrose unloading at the sink may be apoplastic in tissues where plasmodesmata are not fully formed and functional such as embryos or endosperm. In other tissues, sucrose unloading may be entirely symplastic, driven by a steep sucrose concentration gradient from the sieve cell to an awaiting sink cell. The relatively low level of sucrose in the sink cell is maintained by the conversion of sucrose to starch or storage of sucrose in the vacuole.

Once sucrose arrives at the cytoplasm of sink cells, it may undergo any of a number of transformations depending on what type of sink tissue is involved. It may be converted to and stored as starch or fructans in the vacuole if the tissue involved is storage tissue such as roots, fruits, tubers, or seeds. It may also be used for energy or used to supply carbon for different biosynthetic pathways, particularly in growing tissue such as embryos or non-photosynthesizing shoots. In many sink tissues, sucrose may enter multiple pathways, some providing energy and growth components and some being stored.

When sucrose reaches a sink, it is hydrolyzed to glucose and fructose, which are then phosphorylated. These hexose phosphates, primarily glucose phosphate, then move into the amyloplast to be converted into starch. In some storage tissues, particularly potato tubers and the endosperm of cereals, amyloplast-bound starch may comprise up to 80% of the dry weight of the tissue.

One factor limiting improvement of yield in crop plants is not the rate of photosynthesis but rather the translocation of sucrose from source to sink. Translocation rate is governed by mass flow and maxes out at a rate that is species specific. The amount of translocated photoassimilate is directly related to the amount of phloem available. Improving yield may require increasing the amount of phloem in plants as well as improvements to photosynthetic efficiency.

9 Temperature Effects on Growth and Development of Plants

Plants actively grow in a relatively narrow range of temperatures, generally between about 0 and 45°C. Many species have an even narrower range in which they thrive and survival of nearly all plant species does not extend very far above 45°C. Some species can tolerate temperatures far below 0°C due to the remarkable physiological mechanisms of acclimation.

The ultimate cause of cell death at the upper limits of survival is the inability of plants to tolerate the cellular dehydration and the general disruption of metabolism that accompanies high temperatures. At the lower limits, acclimated plant cells can often survive both the dehydration that accompanies ultra-low temperatures and the rehydration that must occur upon thawing. Non-acclimated cells normally survive neither.

There are physiological reasons for the growth responses plants have to temperature and we will explore them in this chapter. We will also explore the physiology of low temperature stress resistance in plants as well as horticultural practices that can influence a plant's stress tolerance.

Temperature and Heat in the Environment

Measuring heat

Temperature is a measure of the heat content of a substance. In this chapter we are mostly interested in the temperature of the plant and its environment which includes the atmosphere and the soil. The temperature of the soil is well buffered because of its mass and water content, thus sudden and dramatic changes in soil temperature don't normally occur. On the other hand, air temperature may fluctuate wildly with huge diurnal and even hourly changes. The fastest temperature drop ever recorded on earth was 27.2°C (49°F) in 15 min in Rapid City, South Dakota, USA on 10 January, 1911 while the fastest rise in temperature ever recorded was 27°C (49°F) in 2 min in Spearfish, South Dakota, USA on 22 January, 1943 (Lyons, 1997). (Other rapid temperature changes are often cited but are not official records of a recognized meteorological authority.) Both are pretty remarkable.

Thermometers

Temperature is most often measured with thermometers, which come in all shapes and sizes, including bi-metal mechanical thermometers, Galileo thermometers, liquid crystal thermometers, and others. Regardless of type, all thermometers have two major components to them: (i) a temperature sensor; and (ii) a gauge or scale. Probably the most common non-digital thermometer is the liquid-in-bulb glass thermometer. This type of thermometer consists of a sensor (the bulb) filled with a liquid (usually alcohol or mercury) that is attached to a glass capillary (the gauge) with an expansion bulb sealing the end opposite the bulb. Enough alcohol or mercury is contained in the bulb and capillary to partially fill the capillary. The rest of the capillary and expansion bulb contain a mixture of nitrogen gas and alcohol or mercury vapor. As the temperature changes, the volume of alcohol or mercury changes and is reflected in the level of liquid filling the capillary that is read from an inscribed scale on the capillary. Of course the thermometer must be calibrated with a thermometer of known accuracy.

Min-max thermometer

A useful thermometer for horticulturists is the min-max mercury thermometer. This type of thermometer records the highest and lowest temperatures experienced by the thermometer between re-settings. The mercury filled glass tube is 'U' shaped rather than the typical linear shape. One arm of the U is the maximum scale and the other is the minimum scale. When the temperature increases, mercury is forced up the maximum scale, pushing a little wire and glass marker along with it. As the temperature

falls, the mercury retreats but the marker stays put, indicating the maximum temperature reached. In addition, the mercury in the minimum side rises, pushing the minimum marker along with it. The scale on the minimum side reads from low to high in a top to bottom direction while the scale on the maximum side reads from high to low from top to bottom like a normal thermometer. Once the temperature begins to rise again, the minimum marker stays put. Both minimum and maximum markers are reset with a magnet. Digital min-max thermometers are also available.

Thermocouples

Thermocouples are also widely used in horticulture for measuring temperature. A thermocouple consists of a sensor made of two dissimilar metals joined together. When the junction of the two metals warms or cools, a voltage proportional to the temperature is generated. This voltage is calibrated with known standards and the temperature of the junction can be displayed digitally. Different combinations of metals called calibrations are available for different temperature and environmental conditions. Thermocouples can be wired in series to construct a very sensitive device called a thermoelectric module which can be used in low-temperature stress-resistance studies. In these applications, the thermoelectric module is sensitive enough to detect the freezing of microscopic-sized droplets of water, for example the freezing of supercooled water in flower pistils of deciduous fruit trees.

Infrared thermometer

Infrared thermometers are also useful to horticulturists. All objects emit infrared radiation in proportion to their temperature, and an infrared thermometer detects this radiation and converts it into a digital temperature reading. Infrared thermometers are useful in situations where thermocouple sensors, digital electronic sensors or individual thermometers cannot be used to measure the temperature of an object. For example, infrared thermometers are useful for measuring plant canopy temperatures to provide an indirect measure of water stress.

Temperature scales

In order for the temperature readings to be useful, uniform scales must be used in describing measured temperatures. The International Temperature Scale of 1990 defines measurements of temperature from 0.65°K (–272.5°C, –458.5°F) to approximately 1358°K (1085°C, 1985°F). Most of us are familiar with the Celsius (C) and Fahrenheit (F) scales and equate our perception of heat relative to known values of water states at various temperatures. Water freezes (or more precisely, melts) at 0°C (32°F) (cold perception) and boils at 100°C (212°F) (heat perception). Actually, humans begin to perceive that something is hot at a temperature of about 30°C and cold around 5°C. Hot and cold are subjective terms, each individual has their own idea of cold and hot.

Movement of heat

Heat moves as electromagnetic energy, mostly in the infrared region of the spectrum, from an object at a higher temperature to an object at a lower temperature. It can move directly from one object to another or it can move from one object to another by passing through a gas or liquid. Changes in the heat content of an object are reflected in changes in temperature, which we can feel or measure. While heat movement can be explained using laws of physics, heat transfer will be discussed here in relation to its importance in horticulture.

Radiation

Radiation is the movement of heat between two objects that are not in contact with each other. The heat is transferred directly through the air in a straight line from a warmer object to a cooler object, with no change in the air temperature. The best example of this is when you go outside on a cool sunny day and feel quite warm standing in the sunshine. Heat energy is radiated from the sun to your body, and even though the air stays cool, you are warmed. Another example of this is when you sit close to a burning fire. The air between you and the fire is cold, but you intercept heat moving away from the fire and you are warmed.

Conduction

Conduction is the movement of heat through a solid body. The heat transfer occurs due to molecular agitation within the solid body, but the body itself doesn't necessarily move. A good example of this is when you stir a hot liquid with a metal spoon, the spoon heats up and you eventually feel it.

The molecules of the spoon at the hotter end are more agitated and have more energy than those at the cooler end. This energy is passed from more agitated molecules to less agitated molecules and the result is detected as a transfer of heat from the hot liquid to your hand.

Convection

Convection is the movement of heat in currents through a fluid such as air or water. The energy is transferred by actual motion of the fluid away from the source of heat. The fluid heating up over a source of heat expands and becomes more buoyant than cooler fluid around it and moves away from the source of heat. Cooler fluid is denser and less buoyant thus replaces the heated fluid. The entire volume of fluid exhibits currents of moving material as heat is transferred from the source of heat to the liquid or gas. An object placed in the fluid can be warmed by heat transfer from the source through the fluid. Think of potatoes in a pot on the stove. Heat from the stove warms the water in the pot which transfers heat energy to the potato, and voilà, dinner!

A horticultural example

Let's put this all together and evaluate heat movement in a typical production field. During the day, radiation from the sun warms the earth. As the soil, plants, and other objects in the field absorb the incoming solar radiation, they heat up and begin to pass some of that heat energy into the air via conduction. Once the air is heated by conduction, it begins to rise and is replaced by cooler, less buoyant air. Convective currents develop and heat that was captured by solid objects by absorbing solar radiation and passed into the air via conduction moves farther into the atmosphere via convection.

While the soil and other objects are absorbing radiation from the sun, they may also be re-radiating energy back out into space. During the day more energy is coming in than going out, thus the air heats up. At night, more energy is going out than coming in, thus the air cools. In addition, the air passes heat to the soil and plants via conduction and the air cools. As more heat is radiated to space by the soil and plants, more energy can be absorbed from the air and it gets cooler. Water vapor and CO_2 may absorb or reflect some of this outgoing radiation before it escapes into space, trapping the heat near the earth's surface. This is the well-known greenhouse effect. A cloudy night is particularly effective in trapping heat energy near the surface, thus it usually doesn't get as cool on a cloudy night as it does on a similar, but clear night.

Inversions

In general, the temperature of the atmosphere decreases with height. As the atmosphere cools at night a significant amount of cold air may accumulate at the earth's surface, resulting in an inversion of the normal temperature gradient from the earth's surface to space. Instead of decreasing with height, air temperature increases with height for some distance upwards before decreasing in a normal fashion. This layer of warmer air is used in some frost-protection methods.

Temperature and Plant Growth

Plant species normally have an optimum temperature range for growth and development, outside of which they suffer reduced productivity and quality. Most plant growth responses to temperature can be explained by looking at Q_{10} values. Temperature effects on growth and development are particularly important in greenhouse production schemes, as temperatures set incorrectly can have disastrous effects on the crop in question. First let's review the concept of Q_{10}.

Q_{10}

The Q_{10} value is the rate at which a biological reaction changes with a 10°C change in temperature. Many biological reactions have a Q_{10} of 2.0, meaning that the reaction rate doubles for each 10°C rise in temperature. Conversely, the reaction rate is halved with each 10°C decrease in temperature. Many chemical reactions and biological processes have published Q_{10} values. In addition there is a temperature range for every biological reaction, outside of which it will not occur. Thus any reaction has a curve which illustrates the relative rate at which it proceeds from 0 at a minimum temperature to 100% at an optimum/maximum temperature.

Each chemical or biochemical process has a temperature optimum which differs among species. Some species grow well at one temperature while others grow well at a very different temperature. This is because the temperature optimum for life processes evolved with species in their climate of origin and adaptation.

Suppose you were growing a cool-season greenhouse crop with a temperature optimum of 15°C. Photosynthesis, respiration, and most other growth processes are optimally balanced in a narrow temperature window around 15°C and all have a Q_{10} for our discussion. Suppose a faulty thermostat kept night temperatures 5°C higher than they should have been for this crop. You notice that your crop looks 'worn out' and you know that something is wrong (but remember, you don't know that the thermostat is broken). Fertility, light, photoperiod, and pest control all seem in order. So what's the problem? During the day, this crop has a hypothetical optimum photosynthetic rate of 10 (units don't matter here). Respiration occurs at a rate of 3 during the day and 4 at night for a total of 7, leaving you with a net of 3 photosynthetic units per day for growth, development, or storage. If the night-time temperature is 5°C above optimum, then respiration will occur at a rate of 8 units per night (1 (original respiration rate) + 1 (for an increase of 5°C and a Q_{10} of 2.0)). The daily balance under these conditions is 10 units of photosynthesis – 11 respiration units (3 daytime + 8 night-time respiration units) or a daily loss of 1 unit! Over time this deficit in photosynthetic products is apparent as reduced growth and poor quality. And all because the thermostat is off by 5°C.

A similar negative outcome would arise if you reduced the night temperature of a warm greenhouse crop in an attempt to save energy. A 5°C decrease in night-time temperature may not seem like that much and you might think reducing the night-time temperature would reduce respiration thereby making more photosynthetic products available for growth and development. However, reducing the night-time temperature has moved the crop away for its optimum temperature for growth and development as well. Reduced night-time temperatures of warm-season crops interfere with basic metabolism of growth, resulting in unacceptable production. Optimum growth temperatures are determined for a reason. It often takes several weeks after exposure to less than optimum conditions before symptoms appear. If optima are not adhered to, yield and quality will suffer.

Examples of the Q_{10} effect in horticulture

NIGHT TEMPERATURE AND FLOWER COLOR Flower color is often enhanced by decreasing the night-time temperature to just a few degrees cooler than optimum. Growers often do this a week or so before the end of the production cycle for bedding plants to improve quality and salability. By reducing night-time temperature, respiration is reduced just enough to allow more photosynthates to be used for anthocyanin production. Care must be taken not to decrease the temperature too much.

EASTER LILIES Careful timing of Easter lily growth is imperative for successful production. Plants reaching their prime too soon are not acceptable. This is especially troublesome in warmer climates where you're trying to grow a cool-season crop in a greenhouse in mid- to late spring when greenhouse temperatures can soar. If plants are growing too quickly, they can be placed in a cooler at 2°C for up to 20 days to slow their growth with no ill effects.

FALL BLOOMING MUMS Chrysanthemums are another seasonal flower crop where timing of bloom is critical. Consumers don't want them when it's still summer-like, but want them as soon as the weather turns cool. This transition in demand is often abrupt and mums may not be in full bloom when they are needed most. Increasing the night-time temperature can hasten bloom when needed. Again, care must be taken not to warm it up too much, as advanced senescence might occur or plants may bloom too soon.

Thermoperiod and DIF

Just as different proportions of light and dark in a 24 h cycle can elicit various responses in plants, so too can differences in the distribution and duration of temperature exposure during the same cycle. Probably the most well-known thermoperiodic response in plants is the DIF response.

DIF is the difference between the day temperature and the night temperature. If days are warmer than the nights, a positive DIF (+DIF) exists, and if the night is warmer than the day, a negative DIF (-DIF) exists. Stem elongation is enhanced with a more positive DIF, and plants remain short statured if the DIF is around zero or negative. By controlling DIF, growers can manipulate the size of their plants, but only to the extent that a species responds. Some species exhibit a large response to DIF and their height can be readily manipulated with DIF. These species include Easter, Oriental and Asian lilies (*Lilium* spp.), *Dianthus* spp., *Chrysanthemum* spp., tomato (*Solanum lycopersicum*), poinsettia

(*Euphorbia pulcherrima*), green bean (*Phaseolus vulgaris*), *Salvia* spp., watermelon (*Citrullus lanatus*), *Celosia* spp., sweet corn (*Zea mays*), *Fuchsia* spp., *Impatiens* spp., *Portulaca* spp., *Gerbera* spp., *Petunia* spp., snapdragon (*Antirrhinum* spp.), geranium (*Pelargonium* spp.), and rose (*Rosa* spp.). Species with little to no DIF response include squash (*Cucurbita* spp.), platycodon (*Platycodon grandiflorus*), French marigold (*Tagetes patula*), tulip (*Tulipa* spp.), hyacinth (*Hyacinthus orientalis*), *Narcissus* spp., and *Aster* spp. In species that respond to DIF, the response is observed when the plant is normally undergoing significant stem elongation. If a grower knows the crop growth characteristics well, they can time the DIF to occur only during the period of most significant stem elongation and not the entire growth cycle.

In general a DIF of -5°C is sufficient to induce shorter internode length and therefore shorter plants. If the DIF is too negative, undesirable responses such as chlorosis may occur. In addition, growers need to be cautious when using DIF as a method of growth regulation since the rate of crop development is affected by temperature as well. Any DIF treatment that results in an increase in the average daily temperature is likely to result in accelerated crop development and any treatment that reduced the average daily temperature would retard growth and development.

In order to achieve a negative DIF, significant greenhouse heating at night is needed. However, lowering the greenhouse temperature below the night temperature for 2 h at sunrise (which creates a negative DIF) is just as effective as maintaining the negative DIF with heating for the entire night. This procedure is called the 'cool morning pulse'. It reduces the need for excessive heating during the night to maintain a negative DIF.

The DIF response may be a response to gibberellin production. Warmer days and cooler nights (+DIF) often stimulates internode elongation by enhancing gibberellin synthesis or action.

The Growing Season

Growing degree days (GDD)

Just as measuring the daily accumulation of light provides information regarding crop productivity, a plant's temperature history provides useful information in many crop production schemes, especially in field-oriented crops where temperature cannot be controlled. Since growth and development of both plants and plant pests (insects, disease organisms, and weeds) are all dependent on temperature, life stages can be monitored and modeled using growing degree days (GDD), also called heat units, a measurement of heat exposure. A GDD is defined as:

$$GDD = \frac{(Daily\ \max T^{\circ} + Daily\ \max T^{\circ})}{2} - Base\,T^{\circ}$$

GDD can be calculated using either degrees Celsius (°C) or Fahrenheit (°F) as long as appropriate conversions (5 GDDC = 9 GDDF) are made when consulting reference accumulations for specific stages of development. *Base T°* is the temperature at which growth commences in a species. It is often set to 50°F (10°C) (as in this example) but may be set lower or higher depending on the crop.

Modified GDD were adopted about 40 years ago to set limits on the minimum and maximum temperatures considered. All temperatures <10°C (50°F) are set to 10 (50) and all temperatures >30°C (86°F) are set to 30 (86). Therefore the maximum number of GDD possible every 24 h is 18 if using the Fahrenheit scale (i.e. assuming *Base T°* = 50°F, maximum GDD is [(86 + 50)/2] – 50 = 18) or 10 if using the Celsius scale.

Accumulated GDDs are calculated by summing daily GDDs over the growing season, starting the accumulation at some defined point, called the biofix, which might be at the fulfillment of the chilling requirement (covered later in this chapter), full bloom, day of seeding, or even a specific calendar date. Phenology models have been developed for specific crops and pests to help growers make management decisions based on crop or pest growth rather than calendar date.

GDDs are not only useful for monitoring growth stages of plants, insects and disease-causing organisms, but they are also useful in determining crop potential in local climates. If an estimate of GDD accumulation is available at a particular location, crops which may not accumulate enough GDD in a growing season can be avoided while those suited for the climate can be trialed. The GDDC requirements for some key developmental stages of several important world crops are presented in Table 9.1 (Neild and Newman, 1986; Miller *et al*., 2001; Kumar *et al*., 2008).

Another use for GDD is in timing herbicide applications for controlling annual weeds. The base

Table 9.1. Average GDDC (growing degree days calculated using degrees Celsius) needed to reach key developmental stages for four world crops.

Stage	Soybean (*Glycine max*)	Stage	Barley (*Hordeum vulgare*)	Wheat (*Triticum aaestivum*) (Hard Red)	Stage	Corn (*Zea mays*) mid-season hybrid
Seedling emergence	75	Seedling emergence	130	140	Seedling emergence	200
Unifoliate leaf	225	First tiller	330	400	Tassel formation	610
Flowering	978	Stem elongation	520	630	Ear formation	870
First pod	1150	Anthesis	840	850	Anthesis	1400
Final leaf	1430	Seed fill begins	1040	1120	Seed fill begins	1660
Seed mature	2400	Seed mature	1400	1600	Seed mature	2700

temperature for GDD calculation in these cases is usually adjusted to 2°C to reflect the growth of many early season weeds at lower temperatures. As in all examples using GDD, the biofix must be known in order to calculate GDD accumulation.

GDDs are used to time plantings for production of sweet corn (*Z. mays*) and peas (*Pisum sativum*) for processing. A steady supply of product is needed to keep the processing plant running through the season. The best way to ensure that there will be few gaps in production is to plant sequentially based on GDD accumulation.

Predictions of disease or insect outbreaks and the most appropriate times for specific control measures can be based on GDD accumulation.

The progression of bloom in fruit crops can be monitored using GDD accumulations in anticipation of the possible implementation of protective measures against frost injury during bloom. Additionally crop progression towards maturity can be monitored for many fruit crops, especially fruit trees, to schedule labor for harvest. Grape (*Vitis* spp.) harvest can be predicted based on GDD accumulation and the information combined with sugar and acid levels to help determine the best date for harvest.

High temperature stress

In many regions of the world heat stress caused by short-term or constantly elevated temperature results in significant reductions in yield and harvest quality. In general, heat stress is considered as any 10–15°C rise above normal ambient air temperature for a period long enough to induce irreparable damage to any aspect of plant growth and development (Wahid *et al.*, 2007). The rise in temperature is detected primarily by the plasma membrane (Saidi *et al.*, 2011) and is immediately reflected in influx of Ca^{2+} from the apoplast. Factors that influence the intensity of heat stress include: (i) temperature; (ii) duration of exposure; and (iii) the rate of temperature increase. The diurnal average temperature seems to regulate the development of heat stress symptoms over time rather than the absolute daytime or night-time temperature (Peet and Willits, 1998).

The heat-stress threshold is a daily average temperature above which a reduction in growth and productivity is observed. This threshold differs among species and is generally in the 25–35°C range (Wahid *et al.*, 2007). Productivity is often directly reduced with exposure to high temperatures during anthesis or seed fill. Heat stress during anthesis causes sterility and heat stress during seed fill causes photosynthates to be directed towards combating the heat stress rather than filling seeds with storage material.

PHYSIOLOGICAL EFFECTS OF HEAT STRESS Two important direct effects of high temperature are protein denaturation and increased membrane fluidity. Indirect injury is manifest as enzyme inactivation, inhibited protein synthesis, and loss of membrane integrity, which all eventually lead to slow growth, reduced ion exchange, and the production of ROS and other toxins. At moderately high temperatures, death occurs only after long-term exposure. If sudden, excessively high temperatures occur, cellular death may occur very quickly.

Heat stress can greatly reduce photosynthesis. The chemical reactions of photosynthesis in the thylakoid and the stroma are considered the primary site of photosynthetic inhibition (Wise *et al.*, 2004).

In addition, heat stress causes the formation of ROS which in turn cause degradation of chlorophyll a and b, thereby reducing light capture for photosynthesis. Since the plant is fixing less carbon via photosynthesis and respiration increases significantly with heat stress, an extremely negative carbon flow results.

High temperatures can cause leaf and twig scorching, sunburn on any aerial tissue, especially leaves and fruit, and general reduction of growth, all contributing to reduced yield and crop quality. Developmental alterations may also occur. Seeds exposed to abnormally high temperatures may suffer from reduced rates of germination, leading to poor stand establishment and reduced yield. Seeds which do germinate may grow very slowly if at all at high temperatures.

High temperatures may lead to poor seed set as a result of heat-induced sterility. High temperatures cause an extension of tomato styles beyond the anther cone which may result in the lack of self-pollination and result in poor fruit set (Wahid *et al*., 2007). Poor fruit set in tomato may also be the result of assimilate redirection away from reproductive tissue as a response to high temperatures (Kinet and Peet, 1997).

PLANT RESPONSES TO HEAT STRESS One of the first metabolic responses to heat stress is the production of specific proteins called heat-shock proteins. These proteins have a molecular mass from about 10–200 kDa and have chaperone-like activity (Schoffl *et al*., 1999). Chaperones are proteins that are involved in the non-covalent folding and the assembly or disassembly of macromolecules, but are not present when the macromolecules are involved in normal biological functions.

Many plants produce low molecular weight compounds called compatible osmolytes under stressful conditions such as heat stress. Compatible osmolytes are stable and not easily metabolized by the cells and have no effect on cell function, even at extremely high concentrations. Their cellular function is still relatively unknown but perhaps they function to prevent cellular dehydration under stressful conditions as heat stress normally increases transpiration and water demands. Compatible osmolytes include sugars, sugar alcohols, proline, tertiary and quaternary ammonium compounds, and tertiary sulfonium compounds (Sairam and Tyagi, 2004).

Plant hormone levels often respond to heat stress. Abscisic acid (ABA) increases under heat stress. This is not surprising since both heat and water stress often coincide and ABA increases in response to water stress. In some species ABA does not increase during the stress, but rather after the stress is over, suggesting some recovery role for the ABA signal (Maestri *et al*., 2002). Ethylene levels in plants increase with increases in temperature, however, at temperatures normally associated with heat stress, ethylene levels often decline. For the most part, increasing temperatures leads to reduced conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene. The temperature at which this accumulation of ACC occurs is species dependent. Salicylic acid (SA) can induce long-term thermotolerance by stimulating antioxidant activity and enhancing Ca^{2+} homeostasis in the cell. Both gibberellins and cytokinins decline under heat stress leading to reduced growth and yield. Brassinosteroids may confer thermotolerance to some species but not others (Dhaubhadel *et al*., 1999).

Phenolic compounds such as flavonoids and phenylpropanoids increase with heat stress. During heat stress, carotenoids, including xanthophyll, stabilize the lipid phase of thylakoid membranes helping to prevent heat-induced damage. Under high light levels, which often accompany heat-stress conditions, xanthophylls (violaxanthin and zeaxanthin) protect cells from excessive light levels. Zeaxanthin is found on the periphery of light-harvesting complexes where it prevents peroxidative damage to membrane lipids by ROS. Anthocyanins often accumulate in leaves under heat stress, even though levels of anthocyanin often decrease at warmer temperatures in flower petals and fruit. Accumulation in the leaf decreases leaf osmotic potential which may increase leaf water uptake and reduce water loss to transpiration.

Isoprenoids are low molecular weight, volatile secondary plant products. Under heat stress, plants that emit greater amounts of isoprenes from their leaves tend to have greater photosynthetic rates than those whose leaves do not emit large quantities of isoprenoids (Velikova and Loreto, 2005). Isoprenes may react directly with oxygen singlets and protect membranes from oxidation under heat stress. How well a plant tolerates heat stress is often determined by how well it detoxifies ROS like singlet oxygen.

Some species develop heat tolerance, physiological adaptations that diminish the negative consequences of exposure to high temperatures. The first physiological event associated with the development

of heat tolerance is increased fluidity of the lipid bilayer which activates enzymes responsible for the production of antioxidants and compatible osmolytes, both important in the development of heat tolerance. The compatible osmolytes protect the cell from dehydration often associated with heat stress while the antioxidants fight off the oxidative stress caused by the production of ROS under heat-stress conditions. Heat-shock proteins produced under heat stress work in concert with the osmolytes and antioxidants to reduce injury caused by heat stress.

Heat thermotolerance is the ability of a plant to grow and develop with little or no reduction in economic yield with subsequent high temperatures. Thermotolerance often develops within a few hours of an initial, brief exposure to a high, but sublethal temperature. It may also develop after a prolonged exposure to increasingly hotter temperatures via the interaction of heat-shock proteins, ABA, ROS, and SA. How these components confer thermotolerance is not clear.

There have been attempts to induce heat tolerance through foliar applications or a pre-sowing seed treatment with low concentrations of inorganic salts, osmoprotectants, hormones, or oxidants such as H_2O_2. Heat preconditioning of plants has also been attempted. Heat preconditioning has conferred greater subsequent heat tolerance to black spruce (*Picea mariana*), tomato (*S. lycopersicum*), and turfgrasses (Colclough *et al*., 1990; Morales *et al*., 2003; Xu *et al*., 2006). Pre-treating pearl millet (*Pennisetum glaucum*) seed at 42°C before sowing resulted in plants which were tolerant of overheating and dehydration (Tikhomirova, 1985).

IMPAIRED REPRODUCTION CAUSED BY HEAT STRESS Besides reduced photosynthesis coupled with increased respiration, a major reason yield is often drastically reduced with heat stress is that sexual reproduction is impaired at high temperatures. This impairment is reflected in fewer flower numbers per plant or greatly reduced fruit set of existing flowers.

Flowering and fruiting of cowpea (*Vigna unguiculata*) is a good example of just how sensitive the process is. If plants are exposed to 2 weeks of hot nights under long days during the first month after germination, flower production is greatly suppressed (Ahmed and Hall, 1993). The response is not observed under short days. Apparently, heat controls the process and photoperiod regulates the sensitivity to heat. At the next level of development, pod set is severely reduced under moderately high night temperatures due to high-temperature-induced male sterility (Warrag and Hall 1984a, b; Nielsen and Hall 1985a, b). Male sterility was observed only when the high temperatures were experienced in the latter half of the dark cycle and not if the elevated temperatures were applied during the first half of the dark cycle (Mutters and Hall, 1992) and this sensitivity appears to be under phytochrome control (Mutters *et al*., 1989). Pistils were not damaged by high night temperature. Additionally, neither pistils nor male fertility were affected by high daytime temperatures, even those higher than the night-time temperatures.

MANAGEMENT PRACTICES TO REDUCE HEAT STRESS AND ITS CONSEQUENCES One of the most obvious approaches to minimizing economic losses due to heat stress is selecting appropriate crops and cultivars for production. This includes selecting for geographic region as well as growing season. Don't try to grow cool-season crops in hot regions or during the summer in moderate climates.

A good example of crop manipulation for difficult production situations is with lettuce (*Lactuca sativa*). Lettuce is a cool-season crop that can be successfully grown in the spring and fall in warmer climates. If summer production is attempted in hotter regions, results can be catastrophic. Lettuce seed is very sensitive to temperature during the first 12 h of water imbibition. Exposure to temperatures above about 25°C leads to very poor germination and those seeds that do germinate produce weak, unacceptable seedlings (Fig. 9.1). For this reason, direct-seeded summer cropping is not usually attempted in warm to hot climates. Even though there are cultivars that have been developed that are more tolerant of the heat, leaf quality often suffers as leaves tend to be bitter and astringent when grown under hot, long days. Fall lettuce crops are normally seeded in late summer when soil temperatures can still be rather high. One way to minimize heat-induced reduced germination is by sowing seed late in the day and irrigating immediately after sowing. Seeds can then imbibe water from soil cooled by irrigation and seeds will have passed through the sensitive period by the time the soil heats up the next day. Another option is to use primed seed (see Chapter 12, this volume, for information on priming). Primed seed is often more expensive than non-primed seed and all cultivars are not always available as primed seed. Summer

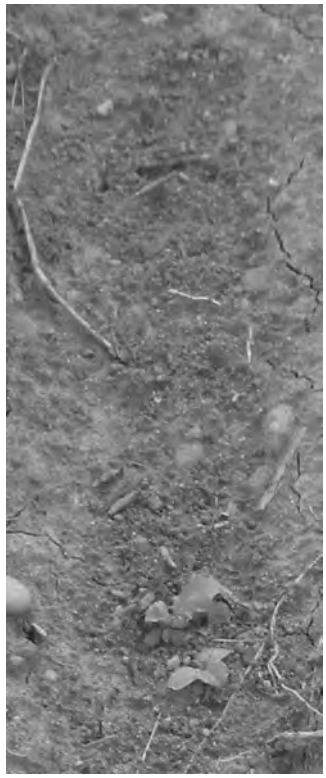


Fig. 9.1. Uneven stand of lettuce due to induced thermodormancy. Raw, non-primed seed was sown when soil temperature was >27°C.

lettuce production under a shaded high tunnel is another alternative. Seed could also be sown in flats of vermiculite, watered and put in a cold room overnight to avoid water imbibition at high temperatures. The additional labor expense of transplanting must also be considered. Ethylene can overcome the inhibition caused by high temperature thermoinhibition of germination (Matillaa and Matilla-Vázquez, 2008), however, no practical seed treatment with ethylene has been developed, and avoidance of thermoinhibition can be achieved by controlled imbibition at low temperatures.

Another management approach to avoiding heat stress is to time production of crops to coincide with the lowest chance of high temperatures during any particularly sensitive stage of development, particularly flowering, pollination, and fertilization. These three stages of development in most species are often extremely sensitive to high temperatures.

Some fruit, tomatoes (*S. lycopersicum*) and citrus (*Citrus* spp.) for example, are particularly sensitive to sunscald and damage by high temperature. Training and pruning techniques can be optimized to provide maximum foliar shading of fruit to minimize injury. In trees where bark temperatures may be excessive, causing injury to the cambium underneath, painting the trunk with a white reflective material can substantially reduce bark and cambium temperature.

As our understanding of how plants tolerate high temperatures improves, stress-resistant cultivars can be developed to reduce the economic losses associated with heat stress.

Dormancy and Crop Production

Many perennial species leave the growing season and enter into a state of visible inactivity most horticulturists call dormancy. This phase of a plant's yearly cycle allows it to survive an often hostile environment in which survival might not normally be possible. While little visible growth is occurring during this period, many things are happening on the cellular level. In fact, regrowth and crop production the following growing season depend on the many physiological changes during the dormant season. When chilling requirements are fulfilled, regular and synchronous flowering occurs supplying the first ingredient for a good crop.

Many buds on a plant remain 'inactive' even during the growing season. It is good that they do not grow, for fields might be overcome with vegetative growth if that were to happen. These quiet buds are also a source of regrowth should something happen to a plant's normal vegetative growth during the growing season, for example some sort of pest infestation which consumes many of the plant's stems and leaves. Plants have evolved a set of finely tuned signaling mechanisms to control bud growth during inhospitable periods or when sufficient growth already exists to support a full crop and further growth is not necessary and might even be detrimental.

Dormancy, rest, and quiescence

The names dormancy, rest, and quiescence have been given to this period of suspended bud growth, resulting in much confusion in the literature and among horticulturists. The problem lies in the specific definition of each term and the inconsistent use of these terms in describing the dormancy phenomenon.

Rather than list the many different definitions that are associated with each term, a more useful approach is to explore the terminology that defines, without confusion, this phenomenon of plant physiology.

A universal nomenclature for dormancy

In the late 1980s, a group of researchers suggested a universal nomenclature for the phenomenon of dormancy that could be used without confusion by laymen and researchers alike (Lang *et al*., 1987). The terms endodormancy, ecodormancy, and paradormancy were suggested as terms that fully described a particular type of bud dormancy in deciduous plants.

Endodormancy describes that type of dormancy that is located within the dormant tissue itself, usually a bud. A physiological condition within the bud makes growth even under optimum conditions highly unlikely. This is the type of dormancy that requires bud chilling before active growth will return in the spring.

Ecodormancy describes dormancy caused by some factor located outside of the plant, usually environmental, that prevents bud tissues from growing. Usually this factor is temperature, but it might also be light or water.

Paradormancy is dormancy caused by a factor within the plant but outside of the affected tissue. A great example of this type of dormancy is dormancy of lateral buds caused by apical dominance of the terminal bud (Fig. 9.2). The lateral buds will not grow, even in an optimum environment, because auxin is being translocated from the terminal bud to lateral buds, preventing their growth. If the terminal bud is removed, the source of auxin is removed and lateral buds will begin to grow.

Fig. 9.2. Raspberry (*Rubus* spp.) lateral buds beginning to grow after removal of terminal meristem and release of lateral buds from paradormancy.

Even though we often describe a particular example of dormancy with one of these terms, the tissue in question may in fact be experiencing more than one type at any given time.

The cell cycle and dormancy

Once released from dormancy, vegetative bud growth requires cell division. Changes in the expression of specific cell-cycle genes have been observed with the onset of bud growth following release from dormancy in many species (Horvath *et al*., 2003). The cell cycle is a series of phases in cell activity involved in cellular reproduction. Cells in the G1 phase are preparing for DNA replication that occurs in the S phase. After the S phase, cells enter the G2 phase to prepare for mitosis in the M phase. Cells in vegetative buds are arrested in the G1 phase just before the S phase, or in other words, at the G1-S phase.

Dormancy breaking results in the release of cells from this arrested phase. Histones and D-type cyclins (CYCD) are involved with this release. Histones are proteins involved in DNA replication and packaging in the nucleus and cyclins are proteins that help regulate the cell cycle by activating kinases, enzymes that phosphorylate other enzymes to activate them. Different CYCDs are expressed when buds are exposed to different signaling agents such as cytokinins, gibberellins, brassinosteroids, and sugar. Once the release from the G1-S phase is signaled by one or more of the above-mentioned agents, cell division moves into the G2-M phase, where it can be arrested due to the action of hormonal signals or by the lack thereof. Movement out of G2-M requires B-type cyclins, which are induced by auxin, cytokinin, and gibberellin.

Physiology of dormancy

Endodormancy

Light and temperature are the two main environmental signals that regulate endodormancy induction and release in plants. When plants perceive either or both signals, their response is the production of a cascade of physiological signals that eventually result in either the induction of or release

from dormancy. These signals are often amplified and transferred within the plant as changes in hormone levels which regulate the many metabolic and anatomical changes that occur.

In some species, such as poplar (*Populus* spp.), birch (*Betula* spp.), red osier dogwood (*Cornus sericea*), and wild grape (*Vitis* spp.), short days alone can induce endodormancy. Other species, such as domesticated grape (*Vitis* spp.), heather (*Calluna vulgaris*), and leafy spurge (*Euphorbia esula*), require both cold temperatures and short days (Chao *et al*., 2007). Few species enter endodormancy utilizing only a low temperature signal.

The short-day response is a response mediated by phytochrome. Endodormancy induction occurs when a sufficiently low P_{fr}:P_r ratio exists after the dark cycle. If the long dark period of a short day is interrupted with red light, dormancy is not induced (Howe *et al*., 1996), and the effect of red light can be nullified if far-red light immediately follows the red light. Cold temperatures enhance endodormancy induced by short days.

A common idea in plant physiology is that bud formation leads to the induction of dormancy or that dormancy leads to the formation of buds. Bud formation and dormancy induction, both initiated by phytochrome-regulated short days are independent processes as mutated plants can become dormant without setting buds (Rohde and Bhalerao, 2007).

Once dormancy is induced, it is maintained by unknown mechanisms. Exposure to low temperature then becomes the key environmental signal leading to a release from endodormancy (Fig. 9.3), however, in many species, this is not a strict requirement as chemical treatments with hydrogen cyanamide can replace the requirement for low temperature exposure (Chao *et al*., 2007). Bud growth may not occur immediately after release from endodormancy due to low-temperature-induced ecodormancy. Once temperatures increase to a sufficiently warm level, ecodormancy will fade and bud growth will commence. After ecodormancy fades, the dormancy-regulating mechanism is reset, probably by increasingly long days and production of dormancy-regulating genes.

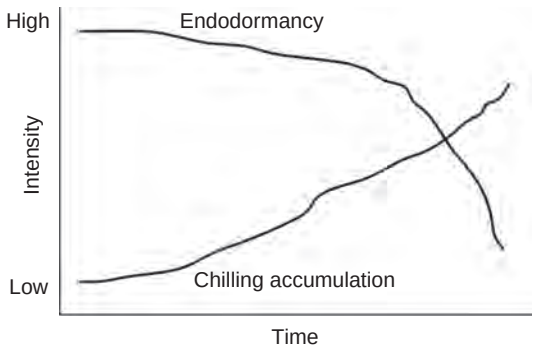


Fig. 9.3. The relationship between release from endodormancy and chilling.

Flowering and endodormancy may be interregulated as genes promoting flowering delay induction of dormancy and genes inhibiting flowering induce dormancy (Chao *et al*., 2007). Senescence is often associated with a decrease in basipetal transport of auxin and this decline in auxin levels associated with senescence may provide a signal for lateral buds to exit paradormancy and enter endodormancy.

Paradormancy

Paradormancy of lateral buds is due to the inhibition of cytokinin production or activity in stem tissue adjacent to lateral buds by auxin. Cytokinin is required for production of cyclin-D. Once auxin levels are reduced enough to allow cytokinin production and passage through the G1-S phase, gibberellins may then trigger the progression into the M phase. Sugar is required for cyclin-D production in some species, but has been shown to inhibit growth of paradormant buds of other species.

Ecodormancy

Ecodormancy is enhanced by high levels of ABA. Factors inducing ecodormancy such as temperature or water stress, lead to elevated levels of ABA.

Chemical messengers

Once the temperature or light signal is perceived by the plant, these environmental signals must be converted to chemical messengers within the plant. These chemical messengers include sugars and the phytohormones. Even though a tremendous amount of research has focused on these messengers, information on how these messengers regulate all three types of dormancy is fairly limited. In addition, while specific regulatory roles are given to different substances, there are myriad interactions among them that occur, both known and unknown.

Sugars (glucose and sucrose) are important in maintaining paradormancy and also the transition from paradormance to endodormancy. Sugars interfere with gibberellin perception and enhance ABA perception (Chao *et al*., 2007). Elevated levels of ABA are associated with endodormancy and ethylene is need for ABA accumulation. Short days inhibit the synthesis of gibberellic acid (GA) and reduced GA levels are often associated with induction of endodormancy. GA is also important for stimulating bud growth following the release from endodormancy, but seems to have limited involvement in endodormancy maintenance.

Chilling and bud endodormancy

We may not know how dormancy release is regulated physiologically, but we certainly have enough evidence that exposure to low temperatures for an extended time is required for release from endodormancy. While it is not a strict requirement since chemical treatments with hydrogen cyanamide can replace the requirement for low temperature exposure (Chao *et al*., 2007), natural release from endodormancy relies on exposure to cold temperatures. Use of endodormancy-releasing chemicals is limited to instances where insufficient natural chilling occurs for adequate bud break and crop production.

Mathematical modeling of the release of buds from endodormancy has been the subject of countless research articles. Much of the work has been with fruit and nut trees. Regardless of which model is used, they all quantify the relationship between low temperature and release of buds, both vegetative and floral, from endodormancy. Such models are extremely helpful tools for horticultural management. Of the many models presented in the literature, three stand out as the most widely used and accepted models for horticultural crops: (i) the chilling hour model (Weinberger, 1950); (ii) the Utah model and modifications (Richardson *et al*., 1974); and (iii) the dynamic model (Fishman *et al*., 1987).

The chilling hour model

The chilling hour model is the simplest model. It assumes that the release of buds from endodormancy proceeds in a linear fashion as plants are exposed to temperatures between 0 and 7°C. Below 0°C and above 7°C, no chilling accumulates and chilling is not reversible. One major drawback of the chilling hour model is that it doesn't account for the negative effect elevated winter temperatures have on chilling accumulation. In addition, the date on which chilling accumulation begins is often arbitrary or based on a specific calendar date. To be effective, a model must account for the chilling a plant receives from the time endodormancy is initiated until it is complete. Experiments needed to determine these points are time consuming and often not performed for different species and cultivars.

The Utah model

In order to account for the negative influence elevated winter temperatures can have on chilling accumulation, the Utah model was developed. This model assumes that chilling accumulation occurs within the temperature range of 1.5–12.5°C. Outside this range accumulation is either negative or zero. The measure of chilling in the Utah model is the chill unit (CU). CU values for different temperatures are presented in Table 9.2. The date chilling begins to accumulate in the fall is determined via an iteration of the model, beginning around 1 August to determine the date on which the greatest number of negative units have accumulated. Normally, in temperature regions, a run of the model for the months August through to November will reveal the date for the start of chilling accumulation each fall. In more tropical climates, this model may continue to accumulate negative chilling units well after November, and a suitable date for chilling accumulation is hard to define or must be arbitrarily set. To overcome this major problem with the Utah model, the dynamic model was developed.

A variation of the Utah model, which is often called the positive Utah model, is when all negative CU values are eliminated from the model. This model

Table 9.2. Chill unit (CU) values for 1 h exposure to different temperatures (after Richardson *et al.*, 1974).

Temperature (°C)	Chill unit value
<=1.4	0
1.5–2.4	0.5
2.5–9.1	1
9.2–12.4	0.5
12.5–14.9	0
15–18	–0.5
>18	–1

provides a better fit to data in subtropical situations; however, the dynamic model is a better choice.

The dynamic model

The dynamic model takes into consideration the impact of high temperatures on chilling accumulation. In this model chilling is accumulated as chill portions (CP) in two separate stages. In the first stage, a hypothetical metabolite is synthesized and accumulates with chilling but may be metabolized and removed from the accumulated pool if temperatures are warm. Once a certain amount of the metabolite is accumulated, it enters a second stage where it is converted with relatively warmer temperatures into a stable, irreversible compound which represents the CP. Stage one is reset to zero to begin accumulating the metabolite again. An adjustment is made to the model for temperatures below 4°C. At these temperatures, only a part of the metabolite is transformed into the CP and stage one is not reset to zero, but rather to a level above zero, the specific value depending on temperature. One CP is equal to 28 h at 6°C or more than 28 h at less effective temperatures (6.7–12.8°C). The mathematics of the model are complex and beyond the scope of this text, however, a spreadsheet is available online (http://ucanr.org/sites/fruittree/How-to_Guides/Dynamic_Model_-_Chill_Accumulation/) for calculating CP values using hourly temperature data. Values for CP accumulation are much lower than those calculated using chilling hours or the Utah model, thus published chilling requirements must be used carefully ensuring that the correct units for chilling accumulation are being utilized.

The dynamic model works well in predicting the stage of endodormancy for those species that have been extensively studied. Rigorous studies are needed to generate the needed information to equate CP to phenological stages for more species (and even cultivars within species) under each climatic zone of interest. As more work is done on this aspect of the dynamic model, probably it will be used more and may become the model of choice for most if not all chilling studies.

Again, the date for initiating chilling accumulation in the fall must be set, and the criteria for setting this may vary from site to site and study to study. A universal phenological stage such as 75% leaf drop should be agreed upon for the initiation of chilling accumulation in the fall.

Determining chilling requirement

When the chilling requirement for a specific cultivar or a lesser known species is not available in the literature, the chilling requirement can be determined experimentally. Besides academic reasons, why would you even want to determine chilling requirement and/or stage of endodormancy? In climates that are marginal for chilling accumulation, it is imperative to have this type of information for scheduling rest-breaking treatments. While estimating chilling requirement demands considerable work, it is not a difficult task.

One assumption of the approach described here, is that typical chilling temperatures will be considered and precise estimates of temperature requirements for breaking rest are beyond the scope of this procedure. In addition, the use of standard chilling temperatures reflects typical field conditions. A physiological marker for establishing the end of rest must be chosen for vegetative, floral, or mixed buds. A standard mark for indicating the termination of endodormancy is growth of 50% of the buds on excised shoots held at an appropriate temperature (15°C) for a specific length of time (3 or 4 weeks) with their cut bases in water. A temperature of 15°C is a good choice since it neither contributes to additional chilling nor negates any accumulated chilling (Dennis, 2003). An alternative indicator would be measuring the length of time it takes for buds to reach a specific stage of development once moved from the chilling environment to the forcing one. A definition of what constitutes growth must also be made, such as greening of bud scales, at least 2 mm growth, etc. One of the models described above must be selected to determine the chilling requirement. If you are located in the temperate zone, either the Utah model or the dynamic model should be used. If you are working in the subtropics, you should use the dynamic model. Recent work suggests that the dynamic model is the best model in all climates (Luedeling *et al.*, 2009). Finally the experimental unit used for making observations must be selected. Small potted trees should be used if available as they represent the closest approximation to orchard trees. Care must be given to small potted specimens in the field to ensure that they do not become desiccated or frozen. Individual shoots with multiple buds or individual nodes with a single bud can be used, but all three have their limitations (Dennis, 2003). Shoot bases should be cut every couple of days to prevent plugging of the xylem.

In general, the procedure is to collect specimens, either potted trees or shoots, periodically from fall through to spring, from the field where they have received natural exposure to chilling temperatures. The samples are then held at a warm temperature (15°C) and periodically the percentage of buds that grow in a specific length of time are determined, or the buds are observed over time and how long it takes them to reach a specific stage is calculated. Determining the percentage of buds that grow in a specific length of time is preferred to observing how long it takes for buds to reach a specific stage as the latter may reflect exhaustion of the buds' food supply rather than a lack of sufficient chilling, especially with cuttings.

Once data are collected, a graph can be drawn with chilling accumulated along the *x*-axis and percentage bud break along the *y*-axis. The chilling needed to reach a certain percentage bud growth can then be estimated. Ideally the work should be repeated for several years and if possible at several locations.

Vernalization

Vernalization is the transition of meristems from the vegetative state to the floral state in response to prolonged exposure to low temperatures. It is required by many biennials including cabbage (*Brassica oleracea* Capitata Group), beets (*Beta vulgaris*), carrots (*Daucus carota*), and winter annuals such as winter rye (*Secale cereale*), and winter wheat (*Triticum* spp.). Vernalization is similar to endodormancy in the sense that both rely on cold temperatures to proceed. However, while endodormancy usually occurs in meristems where cell division has essentially ceased, vernalization requires actively dividing cells to proceed (Wellensiek, 1962). In tissue culture of vernalized *Lunaria biennis* plants, only tissue-cultured plants obtained from actively dividing tissues (meristems) flowered. Plants cultured from non-dividing tissue from vernalized plants did not flower (Sung and Amasino, 2004).

A fascinating aspect of vernalization is that plants retain a permanent cellular memory of their vernalization (Michaels, 2009) such that cuttings of *L. biennis* taken from vernalized plants develop into flowering plants while cuttings taken from non-vernalized specimens develop vegetative plants (Wellensiek, 1962) (Fig. 9.4). When vernalized plants of the biennial *Hyoscyamus niger* are grown under non-inductive photoperiods they do not flower. Even after they are grown for a very long time under non-inductive conditions, the plants 'remember' that they were vernalized and will flower once placed under an inductive photoperiod. This cellular memory is not passed to the next generation since sexually reproduced plants must be vernalized for flowering.

In many species, flowering requires a photoperiodic signal, often a long day, following vernalization. In most of these long-day plants, treatment with GA can replace the requirement for cold treatment. However, in short-day plants, GA cannot replace the cold treatment. Thus the flowering response after vernalization is complex. Many scientists have studied this complex interaction of vernalization and photoperiod, but only recently has a fairly clear picture of what happens at the molecular level developed.

The molecular regulation of vernalization

The incredible plant *Arabidopsis thaliana* has provided enormous clues towards understanding flowering in plants. Besides being a small plant with a rapid generation time and sequenced genome, flowering is controlled by both photoperiod (it's a long-day plant) and vernalization. In many plants, strains of both summer and winter annuals exist in the same species. Many laboratory strains of *Arabidopsis* are rapid-flowering summer annuals. 'Normal' *Arabidopsis* plants are winter annuals that flower under long days after receiving sufficient vernalization. This winter annual trait and the need for vernalization is controlled by a single dominant gene called FRIGIDA (FRI). In addition, the control of flowering by vernalization, requires the FLOWERING LOCUS C (FLC) gene, which represses flowering. When a dominant allele of FRI is present, FLC expression is at a high enough level to inhibit flowering (Sung and Amasino, 2004).

The cellular memory of vernalization

The vernalization process which requires 30–40 days for maximum response, leads to permanent epigenetic repression of the FLC gene for the life of the plant, which allows flowering to occur under an appropriate photoperiod. This cellular memory of vernalization is caused by chromatin restructuring of the FLC which suppresses its expression. The down-regulation of FLC is removed in progeny

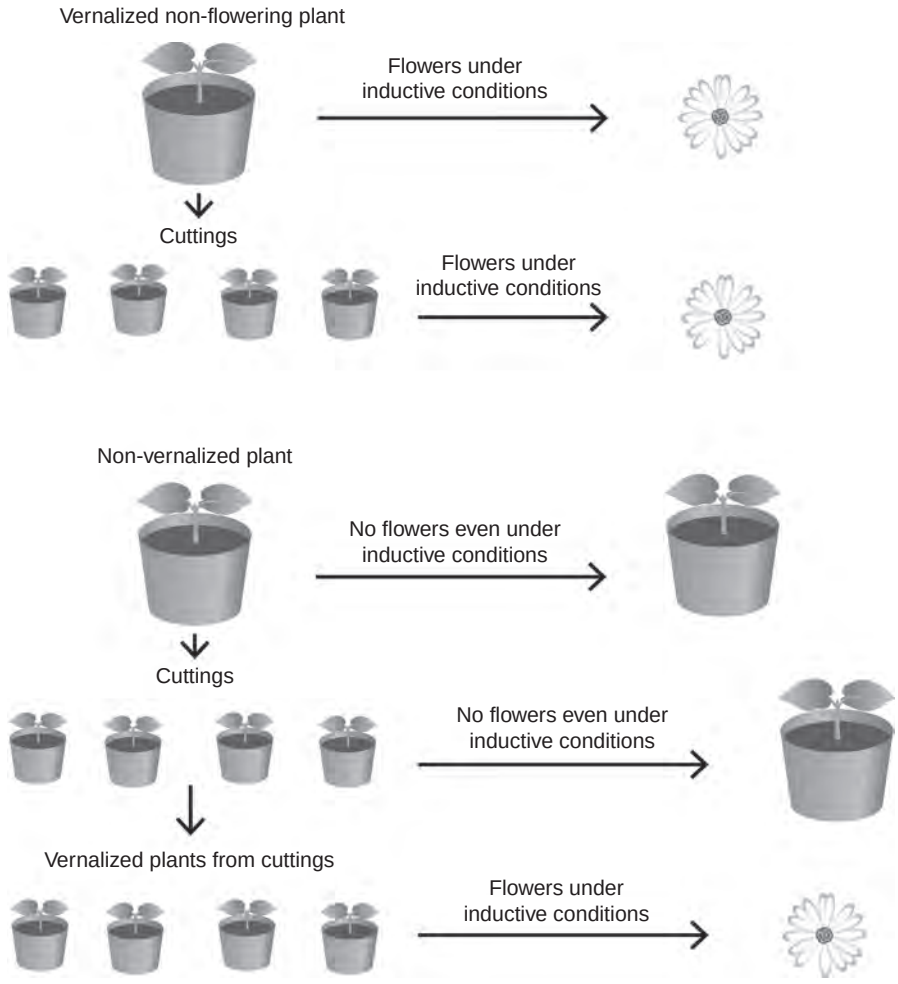


Fig. 9.4. Vernalization is 'remembered' by plants. (Plant and flower symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science, ian.umces.edu/symbols/.)

from sexual reproduction, thus they must experience vernalization in order to flower under long days.

The initial establishment of the vernalized condition is regulated by a gene called VIN3. Expression of VIN3 is induced by exposure to cold, but quickly disappears if the plant is exposed to warm temperatures. The permanent state of vernalization is due to chromatin remodeling of FLC which is regulated by VRN1 and VRN2. VIN3 initially represses the FLC and makes it susceptible to histone modifications triggered by VRN1 and VRN2. (Histones are the protein components of chromatin around which DNA is wrapped. Adding or removing methyl groups from specific amino acids in the histones, confers changes in protein structure which effectively turns the DNA off (adding a methyl group) or on (removing a methyl group).) Repression of FLC by VIN3 is not permanent. Only after methylation of the histones at the FLC locus, which turns off the FLC gene, is FLC permanently repressed, allowing the plant to cellularly remember that it is vernalized (Sung and Amasino, 2004). The FLC gene seems to act by inhibiting the expression of a group of floral activators, also called floral integrators. These are genes coding for proteins that promote flowering. The genes FT and SOC1 are such integrators in *Arabidopsis*.

Flowering after vernalization

The photoperiodic component of flowering in *Arabidopsis* is controlled by the CONSTANS (CO) gene, which is considered a floral promoter. CO expression is low early in the day and increases remarkably 8–10 h after dawn (Michaels, 2009). The protein product of CO peaks about 16 h after dawn (Wigge, 2011) and is stabilized by light and degraded in the dark. Since CO peaks in the light of long days and in the dark of short days the CO protein accumulates and promotes flowering only under long days, since it is not stable in the dark (Michaels, 2009).

CO transcription is regulated by the circadian clock. One protein required for CO transcription is CDF1 which is produced early in the day. It binds to the CO promoter and suppresses CO transcription. Later in the day, two other genes, GI and FKF1, degrade CDF1 and removes the suppression of CO. White, blue, or far-red light promote CO protein accumulation while red light or darkness promote its degradation. The genes responsible for this response have been identified as PHYB, PHYA, VRY1, and CRY2. PHYB promotes the degradation of CO early in the day while PHYA, CRY1, and CRY2 stabilize it later in the day (Michaels, 2009).

Under inductive long days, there is an increase in the activity of the CO gene which in turn activates FT and SOC1. The genes LEAFY and APETALA1, which direct floral organ production at the meristem, are then activated by FT and SOC1. Until FLC is repressed by VRN1, VRN2, and VIN3 genes following vernalization, photoperiod is ineffective in inducing flowering: FLC is repressing the floral integrators! (Amasino, 2005).

Florigen, vernalization, and endodormancy

The protein produced by FT is considered the universal flowering hormone, florigen (Zeevaart, 2008), and evidence indicates that FT is a highly conserved gene across many species (Wigge, 2011). The FT gene has been transplanted into a number of unrelated species, and when overexpressed has led to premature flowering even under non-inductive conditions. Thus FT regulates flowering under both vernalization and photoperiodic control. Not surprisingly, FT is also involved in endodormancy regulation.

In poplar (*Populus* sp.), the onset of dormancy is triggered by short days and cold temperatures with a concomitant down-regulation of FT. Transcription of the CO gene (which activates FT) peaks at the end of a long day or during the night of a short day. As previously mentioned, the product of the CO gene is extremely labile in the dark and only accumulates under long days. Under long days, the CO product up-regulates the FT gene and promotes flowering. When plants are transferred from long day to short day, FT expression quickly decreases and plants set buds. When FT genes are down-regulated using RNA interference (no FT produced) plants are much more sensitive to short-day-induced bud set. However, if FT genes are overexpressed, short-day-induced growth cessation is inhibited (Böhlenius *et al*., 2006) and plants do not set buds (Wigge, 2011). Daylength is sensed by phytochrome, which regulates the CO gene, which in turn regulates the FT gene, thereby regulating both floral and dormancy status of meristems (Rohde and Bhalerao, 2007). After vernalization, there is intense up-regulation of FT expression in embryonic leaves within the bud, suggesting FT's involvement in spring bud growth regulation (Wigge, 2011).

Vernalization and flowering of geophytes

Geophytes are plants in which their perennial buds are located on underground storage organs such as rhizomes, tubers, tuberous roots, bulbs, or corms. Many geophytes are used for their beautiful flowers, both in and out of season. Even though there are about 60 taxa that are regularly used for flowering, the bulk of commercial production worldwide relies on only six taxa: (i) *Tulipa*, 39%; (ii) *Narcissus*, 20%; (iii) *Lilium*, 19%; (iv) *Gladiolus*, 8.5%; (v) *Hyacinthus*, 4%; and (vi) *Iris*, 3% (Gross *et al*., 2002).

Many of these geophytes exhibit endodormancy that must be broken with chilling. In addition, many of them require vernalization for flower formation. Even though endodormancy and vernalization are often discussed separately, the two processes are linked and under similar physiological control. Since many of these species are forced in greenhouses, adequate chilling for breaking endodormancy or fulfilling vernalization requirements is imperative for success. Correct procedures for handling these propagules after harvest in the field until forcing in the greenhouse are easily accessed on the Internet. With respect to chilling requirements, it is absolutely essential to understand: (i) when flower initiation occurs; (ii) how long it takes for

development from initiation to anthesis; and (iii) the temperatures regulating the different phases of flowering. Unlike with tree fruit buds, models are not needed to estimate chilling of geophytes since chilling can be precisely controlled in storage.

Stratification and After-ripening of Seeds

Another important effect of temperature on plant growth and development is its effect on removing dormancy from seeds allowing them to germinate. While there are a number of different types of seed dormancy (including morphological, physical, physiological, morphophysiological, and combinational dormancy; see Chapter 6, this volume) stratification and after-ripening are connected with releasing seeds from physiological and the physiological component of morphophysiological dormancy, allowing them to germinate.

Seed dormancy is an innate characteristic of seed that blocks developmental progress from sexual reproduction to germination. It is controlled by genetics and the environment, in particular, light and temperature. Physiologically it is a very difficult process to measure as it can only be measured by the lack of germination (Finch-Savage and Leubner-Metzger, 2006). Germination is an 'all-or-none' process, it either occurs or it doesn't. Dormancy, on the other hand, is a continuum in the state of readiness to germinate, ranging from not ready at all to fully ready. Dormancy can be controlled by the embryo (physiological dormancy), endosperm, seedcoat, or any combination of the three. Our discussion will focus on physiological seed dormancy, contained within the embryo, that may or may not respond to temperature, GA, or light treatments to remove dormancy and promote germination.

Differences in opinion concerning whether or not a particular factor influences dormancy or germination or both often arise due to differences in how an author defines where dormancy ends and germination begins (Kucera *et al*., 2005; Finch-Savage and Leubner-Metzger, 2006; Tsiantisa, 2006). In this discussion, the viewpoint presented by Finch-Savage and Leubner-Metzger (2006) has been adopted. Any factor that increases the environmental window wherein seeds can germinate is involved in regulating dormancy rather than germination (Finch-Savage and Leubner-Metzger, 2006). For example, many researchers suggest that temperature controls both dormancy and germination while light controls only germination. Since red light alters the seed via phytochrome, rendering it capable of germinating in the dark, it should be considered the last step in breaking dormancy rather than the first step in germination (Finch-Savage and Leubner-Metzger, 2006).

In order for a new seedling to develop, dormancy release and germination cues must be supplied in the proper order. In most cases this consists of a temperature exposure followed by a light treatment. The temperature exposure is usually a long-term process and the response is a slow and gradual shift towards germination. The light exposure is short, as little as a few seconds and the results are instantaneous. As soon as the red light converts P_r to P_{fr}, the switch has been set to the on position and germination can commence.

Plants whose seeds require stratification are said to be in deep physiological dormancy following the classification scheme of seed dormancy proposed by Baskin and Baskin (2004). They require either cold temperatures (subtype a) or warm temperatures (subtype b) during stratification in order to be susceptible to light stimulation of germination and GA will not substitute for the temperature treatment.

The majority of plants produce seeds that are in the 'non-deep physiological dormancy' class. Treatment of seeds with GA can break dormancy, or dormancy can be broken with warm or cold stratification (depending on species) or after ripening. After ripening is storage of seed at room temperature for up to several months in order to break dormancy and promote germination. Physiological changes during the release from dormancy are reflected in seeds germinating at a wider range of temperatures as dormancy is released and an increase in their response to GA and light promotion of germination.

Dormancy is regulated by a balance between GA and ABA in the embryo and the embryo's sensitivity to both hormones. ABA induces dormancy and ABA synthesis occurs in imbibed dormant seeds but not imbibed non-dormant seeds. The ABA production seems to be what maintains the seed in a dormant state. The dormant state is often characterized by ABA synthesis and GA degradation. When GA is applied to dormant seeds, ABA increases, indicating some sort of feedback mechanism to keep the ABA:GA ratio high until dormancy is broken. As dormancy is broken there is a degradation of ABA or a decrease in its biosynthesis with a concomitant increase in GA biosynthesis. GA stimulates germination

and is not really involved in dormancy release. It is the decline in ABA level that signals the loss of dormancy (Finch-Savage and Leubner-Metzger, 2006). During the transition from dormant to non-dormant, there is also a decrease in sensitivity to ABA and an increase in sensitivity to GA. Both cold and light stimulate GA production, gearing the seed up for germination, but until the ABA levels are sufficiently low enough to break dormancy, germination will not occur. The response to cold and light is not really an increase in GA per se since biologically inactive GA9 and GA20 are always formed in the seed (Finch-Savage and Leubner-Metzger, 2006). The increase in GA following cold treatment is regulated by a transcription factor (a protein that regulates the transcription of the message coded in DNA to RNA) called SPATULA (SPT). Prior to dormancy release, this protein prevents expression of the gene responsible for conversion of inactive GAs and GA precursors to GA (Penfield *et al*., 2005).

Different theories have been proposed to explain the after-ripening phenomenon in seeds such as removal of germination inhibitors or changes in membrane structure, however, the physiology of after ripening for the most part has not been documented.

Some species have a dual stratification requirement, for example ash (*Fraxinus excelsior*) (Finch-Savage and Leubner-Metzger, 2006). Warm stratification for 16 weeks followed by 16 weeks of cold stratification are required to remove dormancy and enhance germination. The warm stratification leads to a decline in ABA levels breaking dormancy while the cold stratification increases GA content and stimulates germination. If the cold stratification is given at a constant temperature, germination will proceed slowly, however, exposure to alternating 3 and 25°C leads to hastened germination, but only if the warm period does not exceed the cold period in any 24 h cycle. If seeds receive more warm than cold temperatures during this chilling phase, seeds will enter into a secondary dormancy. This suggests that dormancy breaking is still occurring during the chilling treatment and separating temperature effects on seeds into specific categories is not always justified.

Low Temperature Stress in Plants

In many regions of the world plants may be vulnerable to various forms of injury due to low temperatures. Low temperature stress to plants is often divided into stress caused by low, but above-freezing temperatures (normally called chilling injury) and below-freezing temperatures (freezing injury). Both types of injury can result in substantial economic losses and at times, may be lethal to the entire plant. In this section, both types of low temperature injury and their physiological implications will be explored.

Chilling injury

Many fruits, vegetables, and ornamental crops, particularly those of subtropical or tropical origin, are sensitive to low temperatures (between 0 and 15°C). The more tropical the species, the higher the temperature at which the injury will occur. The injury sustained by exposure to temperatures in this range are very different from injuries due to temperatures below freezing and chilling injury often occurs at a temperature well above freezing. Chilling injury is often associated with injury to the cell membrane, resulting in a physiological abnormality that produces a toxin or fails to produce a necessary metabolite. Freezing injury is often due to direct injury from ice crystals or due to cellular dehydration and an inability to rehydrate upon thawing.

Crops from the temperate zone often experience chilling injury in a much narrower range of temperature (0–5°C). Either the entire plant or a specific tissue may suffer injury. The injury may appear immediately upon exposure to chilling temperatures or may not become evident until much later. Some chilling injury may not be observed until the affected plant or tissue is exposed to warm temperatures.

Chilling injury often reduces or eliminates the market value of many crops, thus this discussion will mainly focus on chilling injury to harvested commodities. Keep in mind that chilling exposure can accumulate, for example in the field, in transit, and in storage, and once a certain intensity is reached, injury will be seen. Crops that are sensitive to chilling injury usually have a short storage life since low temperature cannot be used to slow metabolism and senescence. In addition, symptoms may not be external.

The three main factors determining the extent of injury are: (i) temperature; (ii) duration of exposure; and (iii) plant species. With tropical species, a shorter duration at a higher temperature can lead to substantial injury compared with more temperate crops. Temperate crops can be exposed to lower

temperatures for a longer period before injury will occur. If the intensity of exposure (temperature × exposure time) is low, the damage may be reversible, while at higher intensities, injury is irreversible. In the case of fruits and vegetables, maturity and ripeness also influence injury.

Symptoms of chilling injury

There are many symptoms of chilling injury. A general discussion with examples for injury associated with specific plant parts follows. For symptoms, remedies, and handling procedures for specific commodities, see Gross *et al*. (2002).

Regardless of organ affected, chilling injury is often accompanied by some general symptoms. Desiccation is a classic symptom of chilling injury. This is the direct result of membrane injury affecting its permeability. Chilling injury often alters the lipids in the membrane bilayer which renders it unable to regulate water diffusion out of the cell. Discoloration, water-soaking or pitting is often externally visible. Internal tissues often appear discolored and disorganized, with unacceptable texture or flavor. Injured tissues often experience accelerated senescence and increased decay from microorganisms. Shelf life is often greatly reduced in chilled commodities and they may fail to ripen normally after removal from storage.

FRUITS (BOTANIC) Fruits, in the botanical rather than horticultural context, may suffer substantial chilling injury and a wide variety of symptoms develop. In thick-skinned fruit such as citrus (*Citrus* spp.) and cucumbers (*Cucumis sativus*), sunken areas of discoloration or pits appear on the surface of the skin. In fruit with thinner skins, the symptoms appear as water-soaked areas.

In apples (*Malus domestica*), chilling injury results in low temperature breakdown, brown core, and internal browning. All disorders tend to become more severe with longer storage times. In addition, a cold, wet growing season tends to make susceptible cultivars even more susceptible to chilling injury during storage. Low temperature breakdown is observed as brown discoloration of vascular bundles, flesh browning, and a ring of unaffected tissue just underneath the skin. Brown core is another malady resulting in browned flesh beginning near the core and advancing through the cortex. It is often hard to distinguish between low temperature breakdown and brown core. Internal browning is not browning per se, but rather a distinct graying of the flesh.

Some fruit suffer chilling injury within a very specific temperature range. Apricots (*Prunus armeniaca*) experience 'gel breakdown' when exposed to temperatures between 2 and 8°C, which leads to the formation of water-soaked areas in the flesh that quickly turn brown and become spongy or gel-like. If stored between 0 and 2°C, gel breakdown does not occur.

Avocados (*Persea americana*) can suffer two different types of chilling injury, each caused by different temperatures. Internal chilling injury is caused by exposure to temperatures around 6°C and is characterized by gray-brown discoloration of flesh at the base of the fruit (opposite the stem end) around the seed. External chilling injury, which occurs at temperatures below 3°C, appears as dark, patchy discoloration of the skin.

Banana and plantain (*Musa* sp.) fruit are susceptible to chilling injury at temperatures as warm as 13°C. Symptoms appear on the peel as brown or black streaks along with a grayish cast to the flesh. Fruit may also fail to ripen properly.

Green beans (*P. vulgaris*) experience chilling injury if stored at 5°C or lower. The general symptoms include a general opaque discoloration of the entire bean and sometimes pitting on the surface. At slightly warmer temperatures (5–8°C) rust-colored lesions which are very susceptible to pathogen attack appear on the surface. If held at warmer temperatures to avoid chilling injury, undesirable seed development, yellowing, and desiccation can occur.

ROOTS Jicama (*Pachyrhizus erosus*) roots are crisp, white, and somewhat sweet tasting. Chilling injury may develop after storage at 10°C or lower for as little as 1 week but no injury is observed at roots stored at 12.5°C. Externally, injury appears as decay with flesh discoloration and loss of crispness appearing internally. Roots may even become rubbery in severe cases.

LEAVES AND STEMS Basil (*Ocimum basilicum* L.) is a culinary herb with extreme sensitivity to chilling. If exposed to temperatures lower than about 12°C, severe blackening and necrosis of leaves and stems will occur.

While not necessarily considered a form of injury, potatoes (*Solanum tuberosum*) are very sensitive to storage temperature with profound changes in starch and sugar ratios occurring with

changes in temperature. This can have major effects on the suitability of the tubers for different culinary uses. After harvest, potatoes are cured at 20°C for 1 or 2 weeks to stimulate suberization, wound healing, and to reduce respiration. If cured, potatoes can be stored for up to 1 year. At temperatures above freezing but below 10°C, starch in the tuber is readily converted to sugars which may give the potato an unpleasantly sweet taste and darken during cooking. Tubers for fries or chips are stored at 10–15°C, depending on cultivar. Many 'chipping' cultivars will accumulate excess sugar if stored at <15°C and these sugars readily burn during frying. Most chipping cultivars are stored at 15–20°C which makes them more susceptible to decay and attack by pathogens. Besides irreversible changes in carbohydrate composition at temperatures below 10°C, a mahogany discoloration of the flesh can occur with exposures to 1 or 2°C for prolonged periods.

Asparagus (*Asparagus officinalis*) is harvested for its succulent stems (spears) just as they emerge from the soil. Spears lose their bright and shiny appearance and spear tips become gray after about 10 days at 0°C. Dark spots or streaking near spear tips may occur under severe chilling conditions.

Ginger (*Zingiber officinale*) harvested for its pungent rhizome may lose skin color and experience pitting of skin if held below 12°C. Internal breakdown may also occur.

FLOWERS Flowers with a tropical origin such as *Anthurium*, bird of paradise (*Strelitzia* spp.) and ginger (*Z. officinale*) may suffer chilling injury at temperatures below 10°C. The symptoms of injury include water-soaked petals followed by blackened leaves and petals. A major exception to this general rule is orchids (family *Orchidaceae*). Many orchids are not sensitive to chilling injury and they can be safely stored between 0 and 12.5°C, depending on cultivar.

FOLIAGE PLANTS Many foliage plants are injured by temperatures below 15–18°C. To prolong usefulness, many foliage plants are shipped at 10–13°C, but this is the absolute lowest that foliage specimens should experience as some injury may occur at these temperatures. Many growers acclimate plants before shipping by reducing water, light, fertilizer, and temperature for 2–4 weeks before shipment. This makes the specimens more tolerant of environmental stresses likely to be encountered on their journey from grower to consumer.

FLOWERING POTTED PLANTS Chilling-sensitive potted flowering plants that are injured below 10–15°C include African violet (*Saintpaulia* spp.), *Bougainvillea, Browallia*, Christmas cactus (*Schlumbergera*), *Clereodendron, Crossandra, Cymbidium*, Easter cactus (*Hatiora gaertneri*), *Exacum, Gloxinia, Hibiscus*, poinsettia (*E. pulcherrima*), and *Streptocarpus*.

BEDDING PLANTS AND SEEDLINGS Vegetable and flower bedding plants are often subjected to stressful conditions during transport and storage at retail outlets. Two types of plants are often considered for marketing: (i) plugs; and (ii) finished plants. Plugs are more compact and are easier to ship and store than finished plants while finished plants are often hardier than plugs. Injury to plugs or plants may include minor cosmetic injury, delayed or advanced flowering, stunted growth or even death. There are three general groups with respect to chilling sensitivity:

- The most sensitive species should never experience temperatures below 15°C including balsam (family *Balsaminaceae*), fibrous begonia (*Begonia* × *semperflorens-cultorum*), *Celosia*, celery (*Apium graveolens*), coleus (*Solenostemon*), cucumber (*C. sativus*), eggplant (*Solanum melongena*), *Kochia*, muskmelon (*Cucumis melo*), pepper (*Capsicum annuum*), pumpkin (*Cucumis* spp.), squash (*Cucumis* spp.), tomato (*S. lycopersicum*), *Vinca rosea*, watermelon (*Citrullus lanatus*), and *Zinnia*.
- Moderately sensitive species should never go below 10–13°C including *Ageratum*, aster (family *Asteraceae*), broccoli (*Brassica oleracea* Italica Group), *Browallia*, Brussels sprouts (*Brassica oleracea* Gemmifera Group), cabbage (*Brassica oleracea* Capitata Group), Cauliflower (*Brassica oleracea* Botrytis Group), collards (*Brassica oleracea* Acephala Group), *Centaurea cyanus, Dahlia, Dianthus*, dusty miller (*Centaurea cineraria*), geranium (*Pelargonium*), *Impatiens*, lettuce (*L. sativa*), marigold (*Tagetes* spp.), *Nierembergia*, onion (*Allium cepa*), *Petunia, Phlox, Portulaca, Salvia*, and *Verbena*.
- Relatively insensitive species can be subjected to temperatures as low as 7–10°C without injury, including alyssum (*Lobularia maritima*),

Calendula, carnation (*Dianthus caryophyllus*), larkspur (*Delphinium*), *Lobelia*, pansy (*Viola* × *wittrockiana*), and snapdragon (tall and dwarf; *Antirrhinum*).

SEEDS AND POLLEN Seeds of many tropical species are chilling sensitive and should be kept at a temperature above 15°C. There are no reports of direct chilling injury to pollen. Keep in mind that most species have an optimum temperature range for pollen germination and growth and low temperatures may reduce pollination and fertilization.

Avoiding chilling injury

Obviously the best protection against chilling injury is avoidance of exposure to inappropriate temperatures. Unfortunately the temperature to which commodities are exposed are not always under our control. In the field, nature controls the thermostat. In transit and storage we control the temperature, however, faulty equipment and human inefficiency may lead to exposure of commodities to injurious temperatures.

There are some approaches to reducing chilling injury. Some approaches are universal, others are crop specific. Consult a good reference such as Gross *et al*. (2004t) for specifics. When selecting cultivars for production, investigate whether or not chilling-resistant cultivars are available and whether there are production practices, such as calcium treatments to stabilize membranes, available that might help avoid injury. If chilling conditions occur, minimize how long a product is exposed to the chilling temperature. Also, in some crops preconditioning consisting of stepwise cooling can allow the commodity to adapt to cooler and cooler temperatures, minimizing injury. Intermittent warming of some commodities allows for the metabolic removal of toxins which can reduce injury. Care must be used with intermittent warming procedures since premature softening may occur and decay may increase due to moisture condensation on the product. If possible, try to store fruit that is more ripe, since it often is less susceptible to chilling injury. Controlled atmosphere storage may eliminate adverse effects on product metabolism by slowing metabolic processes down via decreased oxygen availability, for example in nectarines and peaches (*Prunus persica*), okra (*Abelmoschus esculentus*), and avocado (*P. americana*). Be careful with controlled atmosphere storage since some commodities may experience increased chilling injury under controlled atmosphere conditions, for example cucumbers (*C. sativus*), tomatoes (*S. lycopersicum*), asparagus (*A. officinalis*), and citrus (*Citrus* spp.).

Freezing injury

In the previous section plant injury caused by low but above-freezing temperatures was examined. In this section, injury caused by exposure to temperatures at or below 0°C will be explored. Many species are not able to tolerate any exposure to freezing temperatures while others are quite resistant to freezing injury. Still other species exhibit a remarkable avoidance mechanism, supercooling, to avoid injury.

Frosts versus freezes

Freezing injury to plants can be caused by either frosts or freezes. Many people do not make a distinction between the two, since they both cause similar damage. However, the differences between the two are important since whether or not protective measures to avoid injury are successful depend on which type of episode occurs (Perry, 1998).

Radiational frost

A radiational frost is generated locally, on site by cold air generation through radiational cooling. Radiational frosts occur under clear, calm conditions usually during the fall and spring. The depth of the layer of cold air in the atmosphere usually ranges from as little as 10 m up to 60 m. Cold air may drain to lower regions in a field and frost pockets may develop. Generally, the dew point temperature (the temperature at which the air becomes saturated with water and it begins to condense out of the air) is low during a radiational frost. If enough moisture is in the air, visible hoar frost will occur, if not, a black frost (no visible ice crystals) will be seen. Radiational frosts can occur even if the air temperature is above freezing. Object temperatures regulate whether or not frost forms, not the air temperature. With a radiational frost, there is a good chance for successful crop protection.

Advective freeze

An advective freeze is caused by the movement of cold air at or below freezing into a region. It is

often accompanied by windy conditions with or without clouds. The depth of cold air in the atmosphere may range from 150 to 1500 m with no warm air inversion. Cold air drainage does not occur and no frost forms. Crop protection options are extremely limited with an advective freeze.

Evaluating freezing injury

In order to understand the horticultural implications of frost or freezing injury, methods for evaluating injury caused by such stresses have been utilized for many years. Generally tissues are divided into vegetative tissue or reproductive tissue (flower buds, flowers, and young fruit) for damage assessment.

VEGETATIVE TISSUE Visual evaluation of freezing injury to vegetative tissue is relatively easy to do and requires no special equipment. The vascular cambium is the tissue most often evaluated in vegetative tissues. The cambium is evaluated for browning either immediately or 1–2 weeks after the freezing event. The tissue is cut with a razor blade and observations made ranking any injury as none (no tissue browning) to severe (most tissue is brown). It is very subjective, but none the less, it provides a reasonable estimate for the degree of injury.

Another fairly easy assessment of injury involves collecting samples following a freeze, holding them at room temperature for 1–2 weeks and monitoring bud growth. Fewer buds will grow following injury. The assumption must be made that any chilling requirement has been fulfilled prior to forcing after freezing. Late-season assessments can be performed in the field simply by monitoring bud break as growth commences in the spring.

A more rigorous evaluation examines the leaching of electrolytes out of cells following a freezing event. The assumption is made that membrane damage occurs with freezing and the extent of injury can be estimated by how much electrolyte leakage occurs. Non-frozen control samples are handy as a baseline for 'no injury'. Small segments of tissue are incubated in a controlled volume of distilled water for a fixed time at a fixed temperature. The electrical conductivity of the water solution is measured. The same samples are then heat killed in the same water they were incubated in, allowed to incubate for another fixed time and temperature and conductivity again measured. The extent of injury can be expressed as a ratio of conductivity after injury: conductivity after killing and expressed as a percentage.

REPRODUCTIVE TISSUE Evaluation of flower buds or flowers usually involves observing the pistil for signs of injury. In most species, the pistil is either alive or dead, there's no in-between. Thus visual evaluations of pistils following a freeze are very reliable measures of injury. In the bud stage, flowers are cut along the equator and the pistil(s) examined for browning. Live pistils are green. A percentage injury can easily be calculated from pistil counts. Similarly, open flowers can be evaluated, even in the production field. Field assessment of freezing injury is quite common in fruit production regions susceptible to freezing injury. Samples can also be collected and evaluated for growth following a freeze, but visual pistil assessment is quicker and often easier.

SUPERCOOLING AND FREEZING IN PLANTS Supercooled water is water remaining liquid below 0°C. Water droplets in clouds are often supercooled, freezing when they come in contact with aircraft, causing icing. Some plant tissues also contain water capable of supercooling. Water in pistils and xylem ray parenchyma of many species will often supercool, avoiding physical injury caused by ice crystals and the dehydration associated with their extracellular formation. However, if supercooled water in a pistil freezes, it is lethal. This is an important consideration. Supercooling is a freeze-avoidance mechanism, but only to the point at which the supercooled water freezes. The temperature at which supercooled water freezes is called the supercooling point and varies depending on: (i) species; (ii) tissue; (iii) previous air temperatures; and (iv) stage of endodormancy.

It is also important to consider that all pistils, for example, on the same plant or in the same field are not at the exact same stage of development and are not all at the same level of hardiness. If many buds are examined from a population of buds, an average killing temperature could be calculated. This is often expressed as a lethal temperature (LT). LT_n is the temperature needed to kill a specific percentage of the pistils in a population where n is the percentage killed. Values are often calculated for LT_{10}, LT_{50}, and LT_{90}. An LT_{10} would indicate the temperature at which little damage is done, while an LT_{90} indicates the temperature at which severe injury occurs.

In order to understand freezing in plants, an underlying knowledge of water and freezing is necessary. For pure water to freeze its temperature must be at 0°C or lower and a nucleus for ice crystal formation must be present. Nuclei for ice crystal formation might be other ice crystals, dust particles, bacteria with specific proteins on their coats or chemical crystals such as silver iodide. In the absence of a suitable nucleus, water may remain liquid between 0 and –40°C. At –40°C, the spontaneous nucleation temperature of water, water crystallizes whether a nucleator is present or not. Thus water in plant tissues may remain liquid in this temperature range. However, ice crystals form even in supercooled plant tissue at temperatures somewhat warmer than –40°C. The temperature at which the water freezes is called its nucleation temperature.

When a solute such as sugar, salt or protein is dissolved in water, the freezing point of water is lowered in direct relation to solute concentration resulting in freezing point depression. Freezing point depression is not supercooling. The freezing point of water is maximally depressed to –18°C via addition of a solute. Due to concentrations achievable in plant tissues, the freezing point depression observed in plants is generally on the order of 3–5°C, thus most plant tissue freeze at –3 to –5°C. Supercooled plant cells may not freeze until –40°C.

Ice formation in plant cells always begins extracellularly due to the freezing point depression of the cell sap compared with the depression of the extracellular solution. Once formed, extracellular ice draws water out of the cell, concentrating the cell sap even more. In many cases, ice crystals do not puncture the cell membrane, thus the damage done by water freezing is not due to a puncture of the cell membrane. The damage occurs during thawing, as we shall see in a later discussion of acclimation in this chapter.

Supercooling and bud hardiness has been extensively studied in *Prunus*. The water content of the vascular traces and bud primordia and not the whole bud establish the degree of supercooling in *Prunus* flower buds. Other factors such as soluble sugar content (particularly sucrose and sorbitol) are involved since flower buds with similar vascular trace and primordia water content but from different cultivars, do not supercool to the same level (Quamme and Gusta, 1987). The loss of the ability to supercool during the spring is associated with bud development, which is concomitant with increased size, decreased sugar content, and increased water content of the pistil as well as vascular development from the twig to the pistil (Ashworth, 1982, 1984; Ashworth and Rowse 1982; Callan, 1990; Durner, 1990b). As buds exit endodormancy, the ability to supercool is lost. This loss of supercooling ability may be directly related to chilling accumulation and release from endodormancy and not just circumstantially associated with the other changes occurring over time. When CU accumulation is accelerated, so is the rate of loss in the ability to supercool (Callan, 1990). There is a short period during bloom when *Prunus* flowers are frost tolerant, but this period is short, and frost injury can result in significant damage (Andrews *et al*., 1983).

When water freezes, it releases heat that can be directly measured. This property has been used to study freezing in plants with exotherm analysis and differential thermal analysis. Before discussing acclimation and hardiness, let's look at these two methods. Both methods utilize water's heat of fusion for measuring the freezing point of plant tissues during studies of plant cold hardiness.

EXOTHERM ANALYSIS Heat given off during freezing is called an exotherm. When living plant tissues freeze, two freezing events are often detectable via exotherm analysis: (i) the high temperature exotherm(s); and (ii) low temperature exotherm(s). High temperature exotherms are associated with the non-lethal freezing of extracellular water at relatively warm temperatures (0 to –10°C). Low temperature exotherms are associated with the often lethal freezing of supercooled intracellular water at very low temperatures (–10 to –40°C). As previously mentioned, pistils and xylem ray parenchyma are two plant tissues that routinely supercool. The temperature to which they supercool is easily measure by recording tissue temperature over time during a freeze and examining the time-temperature profile for sudden increases in temperature associated with the freezing of water.

The freezing of supercooled water in pistils has been shown to be directly associated with pistil death in many species, including peach (*P. persica*), apricot (*P. armeniaca*), cherry (*Prunus avium*), and *Forsythia*. By analyzing a sample of flower buds during controlled freezing, a clear assessment of the average lethal temperature for buds can be made at any time during the winter. In this way, the effect of

specific horticultural practices on hardiness can be evaluated quite accurately. Do not merely assume that low temperature exotherms are directly linked to tissue death if studies have not been undertaken to establish the fact. Substantial work is required to link the two events. Also, samples must be evaluated for the number of living pistils prior to testing, since dead flower buds may supercool for several weeks after they are dead (Kadir and Proebsting, 1993).

Exotherm analysis is often performed on woody tissue to examine the lethal temperature for xylem ray parenchyma as death of these cells is often correlated with significant injury.

DIFFERENTIAL THERMAL ANALYSIS Differential thermal analysis is similar to exotherm analysis except that the freezing curve of the live sample is compared to a freezing curve for a known dead sample, hence the term differential.

Dormancy, Acclimation, and Hardiness

The ability of plant tissue(s) to resist or tolerate exposure to low temperatures without injury is given the general term 'hardiness'. While some summer annual plants may have a certain amount of hardiness associated with different stages of their growth, hardiness is of particular interest for individuals concerned with winter annuals, biennial, and perennial plants. In this section the process of acclimation, the physiology of cold hardiness and their interaction with dormancy will be explored.

Seasonal aspects of hardiness in plants

As days become shorter and cooler in the fall, winter annuals, biennial, and perennial plants in temperate regions often acquire a resistance to low temperature injury. This process, called acclimation, is linked to another previously discussed survival mechanism, dormancy. The daylength and temperature triggers change a plant's physiology rendering it less susceptible to low temperature injury. Some of the changes include: (i) increased carbohydrate content; (ii) reduced water content; and (iii) alterations to lipid composition of membranes. Changes in membrane lipid composition are accompanied by changes in membrane sugar and water content as well. These changes during acclimation allow the membrane to recover during rehydration following the dehydration associated with freezing. In non-acclimated plants, these membrane alterations do not occur and membranes are not able to accommodate the rehydration during thawing (Gordon-Kamm and Steponkus, 1984). Thus it is normally the rehydration upon thawing that causes the observable damage of a freezing event.

The process of acclimation is a lengthy one that starts in the fall in response to shortened days and cooler temperatures. The combination of the two triggers is advantageous in that daylength changes are constant from year to year while temperature decreases in the fall are often unpredictable and vary from year to year. Even during an unusually warm fall, acclimation will commence due to the photoperiodic signal. Hardiness will continue to increase over time until a plant has reached its minimum hardiness level (MHL). Once the MHL has been reached, that species will be at least that hardy until dormancy ends. Exposure to cold temperatures normally enhances hardiness beyond the MHL. This gained hardiness may be lost with subsequent exposure to warm temperatures, but the level of hardiness will not be less than the MHL regardless of temperature (Fig. 9.5). Once the end of dormancy is reached, hardiness fluctuates with air temperature and may be substantially less than the MHL after even short exposure to warm temperatures. Reacclimation can occur with exposure to cold temperatures. As dormancy release progresses, most plants' ability to reacclimate is inhibited more and more until reacclimation no longer occurs once dormancy release is complete.

Many plants that acclimate in the fall remain quite hardy and resistant to temperature changes during the winter until endodormancy is broken. Part of the acclimation process intimately linked to a plant's tolerance of low temperature is its ability to tolerate the desiccation associated with freezing in plant tissues. In fact, the difference between acclimated and non-acclimated plants is often a difference in desiccation tolerance. Once enough chilling has accumulated to release the plant from endodormancy, plant hardiness can fluctuate substantially with prevailing air temperature. Loss of hardiness is called deacclimation. Deacclimation is primarily regulated by prevailing air temperature. Many species retain an ability to reacclimate with environmental conditions, particularly with re-exposure to colder temperatures. On the other hand, most species reach a point in their development during the late winter and early spring where they are no longer able to reacclimate. After this

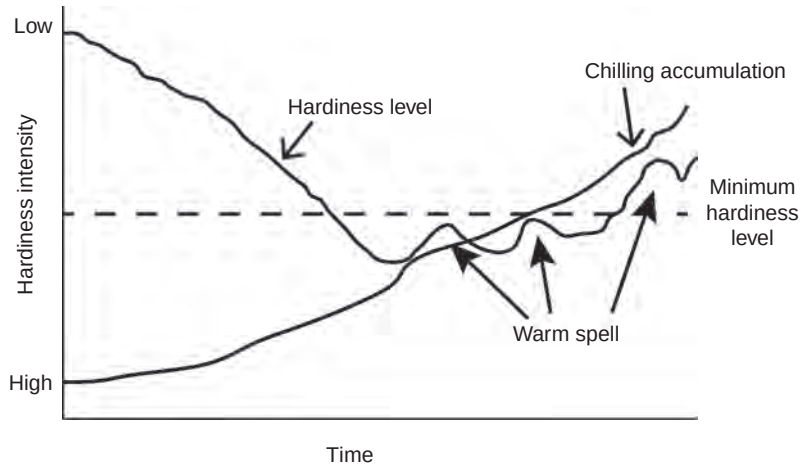


Fig. 9.5. Fluctuations in hardiness in response to prevailing air temperature.

point, exposure to low temperatures is often lethal to at least some part of the plant.

General hardiness of all species is important, however, hardiness of flower buds, particularly those of biennial and perennial fruit crop species, is of tremendous economic importance. Hardiness during dormancy through to full bloom can greatly impact yield in many years when plants are subjected to extremely low temperatures during endodormancy or to frost and freeze events during bloom. The timing of low temperature stress is important. Little injury occurs if the stress occurs when buds or flowers are hardy. However, if it occurs following deacclimation in late winter or spring, results are disastrous.

Maximum acclimation normally requires several weeks to months to occur. Deacclimation may occur within hours of exposure to warm temperatures. In many species, deacclimation proceeds rapidly upon initial exposure to warm temperatures but the rate of deacclimation declines with time. On the other hand, some species deacclimate at a fairly constant rate over time. Reacclimation normally requires substantially longer exposure to cold temperatures, days not hours, to reach the level of acclimation the plant was at prior to deacclimation. The differences in rates of acclimation compared with deacclimation suggest that acclimation and reacclimation require substantial up-regulation of genes for the synthesis of metabolic materials required for the processes (such as sugars, different lipids) while deacclimation results from down-regulation of genes and requires little in the way of metabolites or metabolic energy. Reacclimation is more rapid than initial acclimation probably due to a lack of total deacclimation to pre-acclimation levels. In other words, plants don't deharden all the way upon deacclimation, thus they don't have to start at the very beginning as with initial acclimation. A plant's capacity to reacclimate depends on its previous acclimation and deacclimation history. Naturally acclimated plants may not respond with respect to deacclimation and reacclimation the same as artificially acclimated plants. This indicates that the environment of acclimation directly affects deacclimation and reacclimation more than simply the degree to which a plant is hardened.

Midwinter hardiness and resistance to deacclimation are often independent attributes. A species that is very hardy (*Vitus labrusca*) may deacclimate more rapidly than a less hardy similar species (*Vitus vinifera*) when exposed to warm temperatures. On the other hand, maximum hardiness in rabbiteye blueberries (*Vaccinium ashei*) is observed in cultivars with the greatest deacclimation resistance. Similarly, plants that acclimate more rapidly may not necessarily become hardier than those that acclimate more slowly. It seems that plants that are most resistant to deacclimation gained this capacity through evolution in a climate with large temperature changes during the dormant season (Kalberer *et al*., 2006). Those species with a fairly constant rate of deacclimation evolved in a climate with fairly consistent temperatures during dormancy. Another possibility is that deacclimation resistance

is highest in plants that develop later in the spring or have a deeper ecodormancy following release from endodormancy.

Several mechanisms for cold hardiness are apparent in apple (*M. domestica*). Xylem ray parenchyma and pit tissue avoid freezing injury by supercooling while bark and flower buds do not supercool (Ashworth *et al.*, 1988). Keep in mind that the tissues that avoid injury by supercooling only do so if the temperature does not go below the temperature to which they will supercool. If the temperature goes below this, supercooled water will freeze and injury will result.

Since apple buds do not supercool, they must avoid injury due to desiccation. Hardy buds have a reduced cellular water content, yet somehow maintain enough intracellular water to avoid desiccation injury. Another piece of evidence showing how intimately endodormancy and cold hardiness are linked is gleaned from the fact that during endodormancy, much of the water in buds is bound to other molecules. This bound water helps prevent desiccation injury. As endodormancy is released by chilling, more and more of this bound water is released, probably for metabolic use. As more of the water in a bud becomes free, the bud becomes more susceptible to freezing injury.

As apple cortical cells acclimate, the volume of cytoplasm in the cells increases along with the transient increase in the number of organelles needed for protein synthesis. Starch granules disappear during acclimation, probably being converted into soluble sugars for their cryoprotective effect. Hardiness of apple shoots and buds has been positively correlated with high levels of sorbitol and raffinose family oligosaccharides. Activity of plasma-membrane-associated ATPase increases during acclimation corresponding to the increased metabolic energy requirements associated with acclimation.

When warm temperatures induce growth along with deacclimation, the deacclimation is usually irreversible. This may be due to a reallocation of metabolites away from rehardening towards growth, as well as changes in cell water content and cell structure associated with tissue growth. It could also be due to changes in gene expression involved in growth rather than metabolite and water re-allocation during growth.

Deacclimation is accelerated under long days either through a photoperiodic (relying on responses to phytochrome) or photosynthetic enhancement of growth under long days (Kalberer *et al.*, 2006). During deacclimation, water often moves from frost-tolerant tissues into frost-sensitive tissue. An example of this is observed in *Prunus* (Ashworth, 1992). As flower buds deacclimate, water moves from bud scales into the pistil, rendering the pistil more susceptible to injury. If reacclimation occurs, water moves from the pistil back out to the bud scales, thereby increasing the pistil hardiness. Concomitant with increased water content of susceptible tissues during dehardening is a reduction in soluble sugars, particularly in the raffinose family (stachyose, raffinose). These sugars probably protect membranes from dehydration stress during freezing, thus their reduction during deacclimation is likely to render membranes more susceptible to desiccation stress. However, changes in sugar content may or may not be associated with changes in hardiness. They may simply change due to enzyme activity and its sensitivity to temperature. A specific group of proteins called dehydrins is known to accumulate with acclimation and decline with deacclimation (Kalberer *et al.*, 2006). Dehydrins protect cellular components from the desiccation damage caused by freezing. Blueberry flower buds exhibit an accumulation of dehydrin proteins during acclimation (Muthalif and Rowland, 1994).

Horticultural practices that influence hardiness

Many horticultural practices influence the level of winter injury (Fig. 9.6). In this section, some of these are briefly examined and discussed.

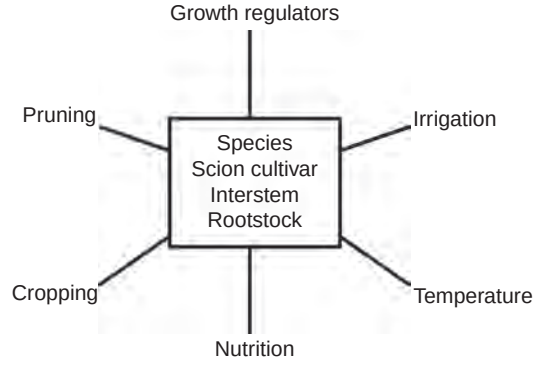


Fig. 9.6. Factors affecting plant cold hardiness.

Pruning

Late summer or fall pruning may stimulate growth of ectodormant buds. Shoots arising from these buds are likely to be very intolerant of low temperatures and significant damage may result. Additionally, active growth may delay acclimation making the entire plant more susceptible to freezing injury.

Pruning stimulates bud growth during endodormancy. Dormant pruning reduces peach flower-bud hardiness by stimulating bud development. Increased bud development leads to increased dehardening during warm weather and reduced rehardening with the return of cold weather (Durner, 1990b, 1995).

Dormant pruning of ornamental specimens and fruit crops is postponed until as late in the winter or early spring as possible in order to assess the amount of winter injury present before pruning cuts are made. In this way, damaged tissue can be removed first, followed by adjustment of specimen form. Additionally, an assessment of potential crop load is made for tree fruit crops by examining flower buds for injury. Since one of the purposes of pruning tree fruits is to reduce excessive crop load, it is important to know what the potential crop load is before pruning commences. In fruit crops particularly susceptible to frost injury, such as peaches and apricots, pruning is often delayed as far into the spring as possible to wait for any 'natural thinning' by frosts to occur before buds are removed with pruning. Unfortunately, growers often have to prune earlier than desired due to the volume of trees that must be pruned in the spring.

Rootstock

Tree fruit crop production often utilizes rootstocks, separate genetically distinct tissues to which the desired cultivar is grafted. Rootstocks influence scion flower-bud hardiness in a number of ways (Brown and Cummins, 1988; Durner and Rooney, 1988; Durner, 1990a; Durner and Goffreda, 1992; Westwood, 1993). Rootstocks can exert a direct influence on flower-bud cold hardiness as hardiness of buds of the same cultivar differ with rootstock after test winters or controlled freezing experiments. Rootstocks also influence the time and rate of bloom in the spring. Buds on rootstocks that induce earlier bloom are more likely to be injured by spring frosts compared with those on rootstocks that induce a delay in bloom. The difference in time of bloom is probably related to differences in chilling and heat requirements of both the rootstock and the scion. The scion may also directly influence rootstock hardiness (Kalberer *et al*., 2006).

Irrigation

Water stress and low temperature stress are related. Conditions that induce water stress are likely to lead to enhanced low temperature stress resistance, if the plant survives the water stress. Plants subjected to water stress during the growing season are often observed to have greater winter hardiness than similar plants that received adequate or excessive water during the growing season.

One method of avoiding injury from frost is by delaying bloom with evaporative cooling using overhead irrigation or under-tree misters. As water that has been applied to trees evaporates, energy is consumed from the surrounding buds with a concomitant decrease in heat units accumulated. In general this method is expensive, requires just the right conditions for use, and often reduces the intrinsic hardiness of buds and flowers, even though their development is delayed (Bauer *et al*., 1976; Strang *et al*., 1980).

Nutrition

Excessive or late-season fertilization can lead to the stimulation of succulent growth which in turn leads to reduced cold hardiness.

Cropping

Since tissue carbohydrate content influences cold hardiness, it is not surprising that crop load can influence cold hardiness (Byers and Marini, 1994). Excessive cropping leads to a drain on the carbohydrate supply of the plant, leaving less available for tissues remaining on the plant after harvest. This carbohydrate deficit leads to reduced cold hardiness of buds and twigs. Light crop loads do not remove excessive amounts of carbohydrates, leaving adequate reserves for cold hardiness to develop in tissue remaining after harvest.

Growth regulators and protectant sprays

Sprays of various materials such as polyvinylpyrolidone K-30, glycerol, ethylene glycol and dimethyl sulfoxide, calcium cyanamid, Frost-Free® (Plant Products, Vero Beach, Florida), and Vapor Gard®

(Miller Chemical & Fertilizer, Hanover, Pennsylvania) have been evaluated for increasing frost resistance or delaying bloom of many tree fruit species. In general, most are ineffective at best and some actually render buds and flowers more susceptible to frost injury (Ketchie and Murren, 1976; Durner and Gianfagna, 1988; Aoun *et al*., 1993).

Probably the most documented and effective treatment to reduce frost injury is the fall application of the growth regulator ethephon to *Prunus* species, particularly peach. A fall application of ethylene increases peach pistil hardiness, delays bloom, and increases yield (Durner and Gianfagna, 1988). Ethephon works by prolonging dormancy and increasing the chilling requirement which delays deacclimation and bloom (Durner and Gianfagna, 1991a). In addition, ethephon-treated pistils exhibit enhanced supercooling and an increased number of pistils that supercooled after deacclimation. Concomitant with this enhanced hardiness were: (i) smaller pistils; (ii) decreased pistil water content; (iii) increased pistil sucrose and sorbitol content; and (iv) slower growth rates during bloom (Durner and Gianfagna, 1991b).

Whitewashing trees delays bloom slightly, but not enough to make it an economic option (Durner and Gianfagna, 1990, 1992). Pre-bloom application of dormant oil may be used in an attempt to reduce heat accumulation and oxygen entry into buds and thereby delay bloom. Such an application may delay bloom but it decreases blossom hardiness which may result in decreased yield (Durner and Gianfagna, 1992).

Crop Protection from Frosts and Freezes

Before embarking on an attempt to alleviate or avoid injury from frosts, it is wise to ascertain whether or not frost protection is feasible and worth the effort. Usually with an advective freeze, protective measures to avoid injury are quite limited. This is especially true if the advective freeze is accompanied by windy conditions (and most advective freezes are). The chances of protecting crops from injury are much better with radiational frosts. Besides the type of freezing event anticipated, consideration must be given to: (i) the specific crop; (ii) its stage of development; (iii) its hardiness characteristics; and (iv) the economic implications involved in a protective attempt. It makes no sense to spend more money than a crop is worth in trying to protect it.

In considering protective measures, two major methods for protection are available, passive and active protective measures. Passive methods are preventative measures taken long before a frost is at hand and involves mostly relatively low-cost, biological and ecological measures that may help reduce the need for active measures. Passive measures include such things as: (i) site and cultivar selection; (ii) cold air drainage management; (iii) plant nutrition; (iv) pruning; (v) methods to delay bloom; (vi) plant covers; (vii) irrigation; and (viii) tree-trunk painting or wrapping. Active measures are temporary and immediate, physical, energy-requiring methods used to replace the energy lost during the frost event and are often expensive and labor intensive. Active measures include: (i) heaters; (ii) wind machines; (iii) helicopters; and (iv) overhead sprinkler irrigation.

Passive methods of frost protection

Site selection

The most fundamental measure one can take to avoid injury from frosts is site selection. If the crop is already selected and a choice of site is possible, an appropriate site can almost always be found with enough investigation. When searching for a production site, talk to local agricultural advisors and growers for their input. When the site is 'preselected', these same individuals can help guide the selection of crop, cultivar, and production methods.

The topography of any site should be evaluated for potential frost pockets and low areas where cold air might drain. This is true at both a regional and a local scale. Regionally, valleys are usually colder than elevated sites, especially during the frost season. Locally, any low spot has the potential for being a frost pocket. An easy way to identify potential frost pockets is to look for locations where ground fog readily forms. Ground fogs due to radiational cooling are most prevalent in the fall. Elevated sites or sites with slopes not directly facing the sun delay spring growth reducing the chance of frost damage.

An evaluation of the soil and its drainage characteristics should be conducted. Sandy, dry soils hold less heat than clayey, wetter soils and therefore crops grown on them are more prone to radiational frosts. Organic or peat soils hold less heat, wet or dry, than either sandy or clay soils, and are therefore not a good choice if frost-sensitive crops are planned for the site.

If weather records are available for the site, they should be reviewed for evidence of frosts that may impact production. If site records are not available, regional climate data are normally always available from the local climate office or from an Internet source. In addition, anecdotal evidence may be gleaned from other growers in the area with regard to frost potential.

Sites where cold air will drain either on a local or regional scale should be avoided even if an effective screen to cold air drainage such as a wooden fence or dense planting to protect the field is planned. Cold air will pool behind the screen which would protect the production field. However, adjacent land parcels are bought and sold, buildings and roads built and demolished, thus the air drainage around any site is subject to change.

Crop selection

Only crops adapted to a specific site should be cultivated if successful production is expected. If inappropriate crops or cultivars are selected for a susceptible site, most attempts to avoid frost injury will be futile. Besides selecting spring hardy crops, care must be given to ensure that the growing season is long enough to allow the crop to mature to reach the point of harvest subsequent to plant acclimation. For example, 'Granny Smith' apple (195 days to maturity) is not an appropriate cultivar in a region with a growing season of 170 days while 'Spur Red Delicious' (150 days to maturity) is a totally acceptable choice.

Canopy trees

Planting an overstory tree is an effective way to provide a small degree of frost protection to the understory crop. Citrus growers in Southern California often interplant date palms (*Phoenix dactylifera*) as an overstory to the *Citrus* crop (Snyder *et al*., 2005). The date trees help hold in some of the heat radiating out into space and the chance of frost is reduced. Several other examples include an overstory of pine trees (*Pinus* spp.) for the satsuma mandarin (*Citrus unshiu*) crop in Alabama and shade tree overstories for coffee (*Coffea arabica*) in Brazil.

Plant health

While it may sound like common sense, healthy plants are more resistant to frost than unhealthy ones. Proper care throughout the rest of the year may add some resistance to frost in the spring. Proper nutrient management, pruning, irrigation, and pest control all support healthy plants. Another aspect of this is that frost-injured plants are often more susceptible to pest pressure, thus turning the problem into a vicious circle.

In general, excessive nitrogen increases plant susceptibility to frost injury by enhancing the growth of succulent tissue. In fact, any management practice that encourages new growth late in the season should be avoided, as new growth is likely to acclimate to a lesser degree than older growth. Two other nutrients often cited for improving frost resistance are phosphorus and potassium. However, there is no clear cut evidence that either improves frost resistance other than that exhibited to well-grown, healthy specimens.

Bloom delay

Evaporative cooling from overhead irrigation can be used to retard the accumulation of heat units in the late winter and early spring, delay bloom and thereby reduce the chances of frost injury. Since the chances of a spring frost decline rapidly over time, even a delay of several days might make the difference between no crop and a full crop. This method of bloom delay depends substantially on the weather in that air is only cooled to its dew point temperature, thus in a humid climate, little cooling below the air temperature might be accomplished with this method. During warm springs in a drier climate delays of up to 2 weeks can be achieved. While this method is included under passive measures, it actually requires substantial grower commitment of time, energy, and money to implement such a venture.

Fall applications of the growth regulator ethephon can delay bloom in peach (*P. persica*) and cherry (*P. avium*) by up to 2 weeks in a cool spring and for several days in a warm spring. An additional benefit of fall-applied ethephon is an increase in the intrinsic hardiness of peach and cherry flower buds both in the winter and during bloom.

Row covers

Synthetic row covers made of spun-bonded polypropylene are often used to increase downward long-wave radiation at night to protect from frost (Fig. 9.7). These covers are lightweight, opaque to

Fig. 9.7. A row cover such as this provides several degrees of protection against frost injury for sensitive species. The amount of protection is determined by weight of the row cover, with heavier weights providing greater protection.

long-wave radiation and allow air, water, and light to pass freely. However, they are expensive and are easily blown about even in light wind and must be secured with sandbags or other heavy weights. Straw offers an alternative; however, it must be removed to allow light exposure. Many other forms of covers are utilized depending on region and availability. Many are relatively inexpensive, however, all materials including straw and synthetic row covers require substantial labor for installation and removal.

Soil cultivation, moisture, and row middle management

When a frost is anticipated, soil should not be cultivated to maximize heat retention during the day and heat transfer to crops at night. Cultivated soil is aerated and holds less heat than non-cultivated soil. If possible, soil should be irrigated prior to a frost event. Water holds a tremendous amount of heat, thus a wet soil will store more heat during the day than a dry soil. That stored heat can then be released at night, reducing the chances of frost injury. Row middles should be mown as short as possible prior to a frost. Excess vegetation reflects solar energy and increases removal of soil moisture via transpiration. Thus crops with row middles of longer vegetation are more likely to suffer from frost injury than crops with short row middles. Grasses and weeds often have a high population of ice nucleation-active (INA) bacteria associated with them, thus mowing overgrown middles might remove some of the INA bacteria. However, bacteria will still be on the mowing litter and many frost-prone species have intrinsic nucleating agents in them. Thus reducing INA bacteria populations does not guarantee a reduced risk of frost. Removing the entire row middle down to bare soil might reduce the chances of spring frost but would make the field more prone to wind and water erosion.

By covering the soil surface with plastic sheeting heat storage during the day is increased. Clear plastic is a better choice than black plastic as more energy is stored in the soil under clear plastic. Wetting the soil before plastic application also improves heat storage. Organic mulches should not be used as they reduce the transfer of solar energy to the soil. However, midwinter organic mulching may provide protection from soil heaving.

Tree-trunk painting

The trunks of deciduous trees are often covered with a wrap or white latex paint to reduce temperature fluctuations on sunny winter days. There can be as much as a 20°C difference in trunk versus air temperature on a sunny, cold day. If the sun is suddenly blocked by clouds, trunk temperature may plummet and bark cracking can occur. Cracked trunks expose tissue to disease and insect pests, as well as creating a wound that the plant must heal. Trunk painting or wrapping has also been reported to delay bloom in apples (*M. domestica*) by a few days.

Insulated trunk wraps are often used in *Citrus* production to provide as much as 8°C protection to injury to young trunks from frosts. It is critical that the air spaces in the wrap are not filled with water during irrigation or rain, as they will lose their protective nature and may also increase the chances of injury when wet. Soil mounding can be used to protect young trunks from injury; however, the level of protection soil mounds afford is quite variable. In addition, disease pressure is often higher with soil mounds compared with trunk wraps.

Controlling INA bacteria

As previously mentioned, many plants have intrinsic ice nucleators, thus controlling populations of

INA bacteria does not guarantee reduced chances of frost injury. The main INA bacteria are *Pseudomonas syringae*, *Erwinia herbicola* and *Pseudomonas fluorescens* (Lindow, 1983). In crops that do not necessarily have internal ice nucleators present, controlling populations of INA bacteria may afford some protection from frost. Populations of INA bacteria can be reduced with bacteriocides or by increasing pressure from enhanced populations of non-INA bacteria. Again, this type of frost control is expensive.

Frost-protecting sprays

While there are many commercially available sprays that claim to reduce injury from frost, no reputable reports exist confirming their effectiveness. In particular, chemicals which reportedly reduce frost injury by preventing desiccation ignore the fact that injury is from the failure of desiccated cells to rehydrate upon thawing, not from transpiration-induced desiccation. In fact, chemicals that protect against freezing often render blossoms more susceptible to freezing injury.

Active methods of frost protection

Some of the methods listed under passive methods of frost protection could be included in this section, especially those requiring substantial labor and money investments. However, this section will be limited to those methods employed on the night of a frost in an attempt to reduce or avoid frost injury.

Wind machines and helicopters

If a radiational frost is the result of a loss of radiant heat from the earth to the atmosphere, what better way to reverse the situation than by bringing some of that lost heat back to earth? This is precisely what wind machines and helicopters do.

Wind machines are essentially large fans operating at about 600 rpm with two or four 3–6 m blades mounted on a tower 10 m above the field floor that rotate around the tower every 5 min while drawing air aloft towards the surface at about a 7° angle (almost horizontal). In order to be effective, the fan must have warmer air (i.e. an inversion) to draw towards the surface. Besides drawing warm air in from aloft, wind machines can blow colder air out of low spots in the field. Wind machines are environmentally friendly except for the noise pollution they cause.

Helicopters push warm air aloft towards the surface, but only if there is an inversion present. They need to fly over the field under protection once every 30–60 min to be effective. In general, helicopters can increase the surface air temperature by about 4°C with a strong inversion. Helicopters are expensive to use and are normally only used in emergencies.

Heaters

Since frosts are the result of a loss in heat energy, one way to combat their occurrence is by replacing the lost energy through field heaters. The heat released by burning any one of a number of different fuels will directly heat plants through radiant transfer and also heat the surrounding air by conduction and convection. For heaters to be effective the night must be calm with an inversion layer above the field. Heaters will often be used in combination with wind machines or helicopters to stir the heated air up and to force it back to the proximity of the crop after it rises in the atmosphere. If the inversion is strong enough, it will act as a blanket, keeping much of the heat in the air surrounding the plants. It is extremely important that heaters are placed correctly and burnt at the appropriate level so that holes aren't punched in the inversion layer creating chimneys in which most of the heat will escape to the upper layers of the atmosphere. Heaters can be units purchased for explicit use in production fields or they may be open fires in the field.

Heaters are expensive to purchase and operate and less environmentally friendly than other methods of frost protection. They are, however, a dependable form of protection for high value crops. Most of the heat released by heaters is in the form of hot gasses which rise and cool until they reach the ambient temperature when it then cools, spreads out, and begins to sink, creating a circulation pattern. Very little heat energy is moved by radiant transfer directly to plants. The energy generally required to prevent a frost is somewhere around 20–40 W/m^2; heater output is usually around 140–280 W/m^2, depending on fuel, burning rate, and the number of heaters involved. Most of the energy released during burning is lost, thus the process is extremely inefficient.

Heaters are more efficient with stronger inversions (low ceiling) because they have to heat a smaller volume of air. More heaters are needed along the edges of a field to account for cold air being drawn in from outside the field area by the rising air above the heated field. Smoke does not contribute to heat release or retention from heaters, regardless of fuel or burner type. Local regulations should be carefully reviewed before purchasing or using any type of heater.

Sprinkler irrigation

Sprinkler irrigation can be used to effectively and economically protect crops from frost. Many growers already have the irrigation equipment, thus there are only time, labor, and fuel investments with this type of protection. Labor is needed to set up irrigation equipment and also to ensure there is no ice build up on the sprinkler heads during operation. Some drawbacks to this method include: (i) fuel and water costs; (ii) labor requirements; and (iii) potential soil waterlogging.

Frost protection with water relies on the same principles regardless of the application method used. Water contains a large amount of heat energy. That heat can be transferred to the soil or to plants, raising their temperature and reducing the chance of a frost. While water adds heat energy to the environment, it can also remove heat energy through evaporation. Thus one must consider the energy added with water application plus the energy lost due to evaporation. Additionally, water releases heat when it freezes and this heat is transferred to whatever object the water is freezing on. When liquid and solid water are commingled, their temperature cannot go above or below the freezing point (0°C) until a complete phase change (all liquid or all ice) occurs. Thus if a film of liquid water is maintained on ice, the temperature will stay at 0°C. This is the key to frost protection with water. If enough water is not being applied or the system is turned off too soon in the morning, no liquid–solid interface will be maintained and damage will occur, often more than if no protection were even attempted. Additionally, if the system is not started soon enough in the evening, evaporative cooling may cause temperatures to drop sharply and cause extensive damage. Evaporation of water at 0°C removes about 2500–2800 kJ/kg from the surrounding environment, depending on whether the evaporation is from liquid water or ice (2501 and 2825.5 kJ/kg, respectively). When the same amount of water freezes 418.3 kJ/kg are released, thus it takes six times the amount of water freezing as evaporating just to break even! This is especially crucial when the air is dry (which it often is during a radiational frost) and considerable evaporation is occurring.

Overhead sprinkler irrigation systems attempt to maintain a layer of liquid water on the ice forming on plant and soil surfaces, and thereby maintain a temperature around 0°C. This system can provide up to 7°C protection under ideal conditions. Overhead systems are used on low-growing crops and larger specimens that can support the weight of ice that builds up during protection. In some cases sprinkling over the structure of covered crops (crops in unheated greenhouses or high/low tunnels) may be employed to keep the temperature inside the structure at 0°C.

Under-plant sprinklers are designed to maintain a layer of liquid water on ice forming on the soil surface and ground cover. It is used on any crop that benefits from heat movement from soil to plant during a radiational cooling event and on crops requiring only a few degrees of protection.

Computer programs are available to assist with decisions regarding on-off times and application rates based on air temperature, dew point, wind speed, and crop being protected.

Surface irrigation or flooding

Supplying water to a field via trench or furrow irrigation provides heat from the water as it cools. Similarly flooding entire fields with water provides protection from frost during radiational cooling. If the water cools enough to start freezing, any standing water will freeze from the top down due to the density properties of water. Water is most dense at 4°C, thus the warmer water will sink as the colder water rises to the surface and freezes. If a layer of ice forms, a barrier of heat transfer from the warmer, deeper water and soil develops and the ice surface and adjacent air temperature can cool to dangerous levels.

Artificial fog

High pressure lines with specialized nozzles in an irrigation system can create an artificial fog to

protect against frost. The small droplets absorb long-wave radiation and re-emit them downwards providing protection from frost. Light wind and high humidity are required for this method to work and generally work best for moderate frost events. The cost of foggers is high but the operational costs are 20% or less than other conventional heating or sprinkler systems.

Controlling INA bacteria

Controlling the population of INA bacteria is not an effective means to reduce frost injury as most woody species have intrinsic ice nucleators that initiate frost formation with or without INA bacteria present (Gross *et al.*, 1984; Ashworth *et al.*, 1985; Proebsting and Gross, 1988).

10 The Soil and its Environment

Soil is the earth's skin. The pedosphere, which includes agricultural soil discussed in this section, is a complex mix of physical, biological, and chemical properties that are integrated into a base on which we grow our crops. Good soil takes years to develop yet it can be destroyed in a matter of minutes with improper management. While too many people refer to the soil as 'dirt', it is far from being something we need to remove with soap and water. Soil is part of our intricate life support system, and itself is a complex natural body supporting a host of biological diversity we rarely see. Soil is a major recycling system filtering water and providing support for several of the major biogeochemical cycles on the planet (carbon, nitrogen, phosphorus, and sulfur) (Or *et al.*, 2011). Rather than focus on many of the topics already covered in most soil texts, this chapter will focus on nutrients and their cycles, fertilizers and their uses, and mulches. Soil basics will be reviewed and the reader is directed to a good soil text such as the one by Brady and Weil (2007) for greater detail in areas of interest.

Soil Basics

Soil is the outermost layer of the earth's surface supporting crop growth. Most of the minerals needed for plant growth are absorbed by plants from the water in soil, called the soil solution. Characteristics of a particular soil and its solution depend on the parent mineral or organic matter material from which it was derived and the actions of macro- and microorganisms and the environment on them over time. In general, the soil is a 50:50 mixture of solids and pore space. The solid portion is mostly mineral (~90%) with some organic matter (~10%). Peat or muck soils may contain from 20 to nearly 100% of their solid component as organic matter. The pore space is occupied by varying percentages of air and water depending on current conditions.

Soil horizons

When considering soils for agricultural production, soil horizons are often discussed. Soil horizons are layers of soil running parallel to the surface. Each layer varies in thickness from less than a centimeter to many meters, depending on location. In some soils the horizons are easily distinguished while in others they flow one into the next. The topmost horizon, the A horizon, includes the mulch (topmost layer including plant debris, decaying organic litter, etc.) and the plow layer (under the mulch). Living organisms such as plant roots, fungi, bacteria, worms, nematodes, insects, and small animals are abundant in this layer of a healthy soil. In not-so-healthy soils, the biology of this layer may be severely compromised. The B horizon is located just below the A horizon and is often called the subsoil. It has characteristics of both the B and the C horizon. The C horizon is the parent material of mineral soils and lies below the B horizon and is usually the deepest layer.

Soil texture

Soil texture describes the relative proportion of particles of various sizes that make up the solid portion of a soil. The largest particles are sand (0.05–2.00 mm diameter), followed by silt (0.002–0.05 mm), and clay (<0.002 mm). Gravel is often part of many mineral soils. A particular soil is classified into one of 12 soil textural classes developed by the USDA (Brown, 2003) and can be determined using the USDA soil texture triangle or obtained from a soil test report (Fig. 10.1). Organic soils are those soils containing a significant amount of plant and animal remains in the process of decay. These soils are classified as mucks or peats, mucks being more highly decomposed than peats. In soils that have a substantial amount of fragments of coarse parent material, such as stones or

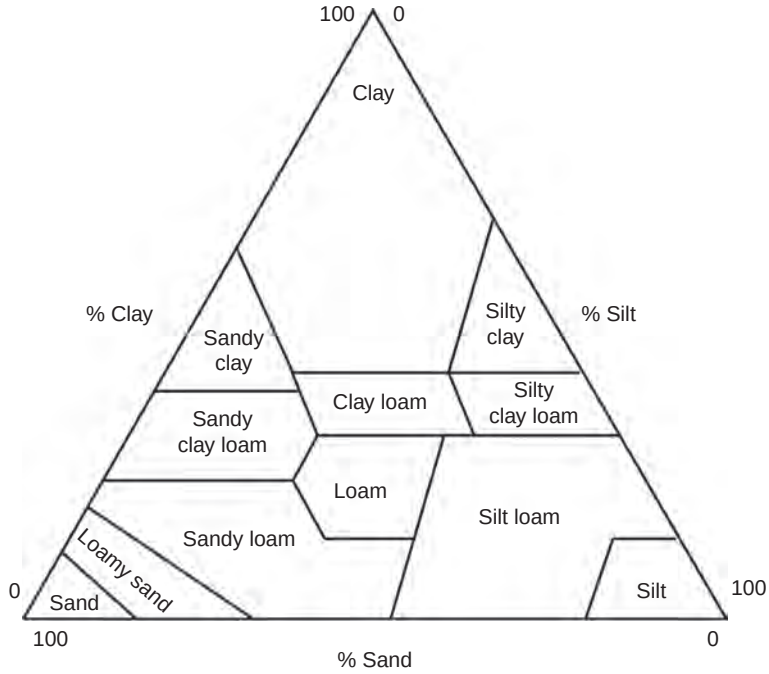


Fig. 10.1. Generalized soil texture triangle with soil texture names based on component percentages.

gravel, their presence is recognized by prefixing a textural class name with an appropriate adjective, such as 'gravelly, sandy loam'.

Soil texture has a profound influence on soil fertility, capacity to retain fertilizers, cation exchange and buffering capacity, and soil permeability and drainage. As the relative amount of clay and silt increase, these soil properties are increasingly affected. Clay- and silt-based soils tend to be more fertile with higher cation exchange and buffer capacities than sandy soils. They also retain more water than sandy soils, but may also be poorly drained if clay or silt amounts are excessive.

Soil structure

While texture refers to the proportions of the different sized solid particles in a soil, soil structure refers to their aggregation or lack thereof. Aggregation in the soil results from physical and chemical processes and takes time to achieve. Good soil structure takes a long time to develop but can be destroyed in a matter of minutes. Working the soil when it is wet is the number one way to ruin a soil's structure. Overworking soil when it is not wet is the other major activity that will destroy soil structure. Organic matter binds the sand, silt and clay particles together, thus the addition of organic matter to soils can improve their structure significantly, especially when the soil is originally low in organic matter. In extremely sandy soil, organic matter may not improve soil structure since the soil lacks structure to begin with. Soil structure combined with soil texture gives a soil its physical properties conducive to plant growth. These properties include water holding and drainage along with nutrient characteristics. It is much easier to correct nutrient deficiencies in a soil than to quickly improve a soil's structure.

Soil bulk density

Soil particle density is the mass of solid particle per unit volume and is generally around 2.6 g/cm^3. Soil bulk density is the mass of dry soil per unit volume, and unlike particle density, it takes into account pore spaces in the soil that are due to the soil's structure. Thus soil bulk density varies with structure while particle density does not.

Well-aggregated soil has a lower bulk density than poorly structured soil. When soil bulk densities are too high, root penetration and water infiltration is reduced resulting in poor plant growth. Bulk densities generally range from 1.0 to 1.8 g/cm^3 in well-aggregated soil.

Soil color

Soil color, especially subsoil color, can reveal good information concerning long-time water drainage of the soil. Well-drained soils often have reddish-brown subsoil while the subsoil of poorly drained soil is often mottled and gray. Soils with fair drainage often have pale yellow subsoils. Surface soil color varies tremendously with parent material and amount of organic matter.

Soil organisms

Most soils are teaming with life. Besides plant roots, there are many small to microscopic organisms living at and beneath the soil's surface that have a tremendous influence on plant productivity. The distribution and activity of soil organisms is related to food supply, temperature, soil pH, moisture availability, tillage practices, and the use of pesticides.

Arthropods

Arthropods are animals that have an exoskeleton such as insects, crustaceans, and arachnids. Arthropods in the soil shred organic matter, stimulate microbe activity, and recycle nutrients. Some arthropods such as millipedes and termites are shredders, chewing up dead plant material. Herbivores in the soil like mole crickets feed on live plant roots. Both shredders and herbivores can become a pest if their population gets too high. Finally, predator arthropods such as spiders and centipedes feed on other arthropods, helping to control their population, or feed on fungi and bacteria.

Bacteria

Bacteria are single-celled, microscopic organisms that are important in nutrient recycling, causing or suppressing diseases, and removal of toxins from polluted soils. Bacteria are often categorized as pathogens, decomposers, mutualists, or chemoautotrophs. Pathogens cause disease and decomposers break down organic matter in the soil. Mutualists such as *Rhizobium*, live in symbiosis with plants such as beans, clover, and alfalfa, providing nitrogen to the plant and receiving carbohydrates in return. Chemoautotrophs are bacteria that can utilize nitrogen, sulfur, or iron compounds for energy. They are often used to clean up pesticide or oil spills from a soil.

Earthworms

While not a required component, earthworms often indicate a healthy soil. Earthworms help aerate the soil by their burrowing action creating macropores in the soil that enhance root growth, gas exchange, and water infiltration. They are the primary consumer of organic matter in a soil and as such are responsible for decomposing plant materials and recycling nutrients. Earthworm populations and their activity in the soil is enhanced with organic matter and reduced with cultivation and pesticide use. Earthworm casts improve soil structure by enhancing soil aggregation and are a rich source of nutrients, especially nitrogen. The nitrogen content of earthworm casts is directly related to the nitrogen content of the organic matter consumed, with casts containing approximately 70% of the nitrogen in the consumed organic matter.

Nematodes

Nematodes are microscopic, non-segmented worms that live in the soil. Some feed on bacteria, fungi, and algae in the soil while others are plant or animal parasites. Nematodes help recycle nutrients in the soil and can either suppress or cause plant disease, depending on their feeding habit. Predator nematodes tend to suppress disease by ingesting disease-causing organisms. Those that feed on plant roots often transmit viruses that can cause disease.

Soil fungi

Fungi are abundant in most soils. They can be single celled like yeast or multi-cellular with an elaborate network of hyphae. They are important in nutrient recycling, decomposition of organic matter and can either cause or suppress plant diseases. An important group of fungi important in nutrient acquisition from the soil are the mychorrizae.

Soil protozoa

Protozoa, mobile single-celled organisms that are larger than bacteria, are important for nutrient recycling and regulating soil bacteria populations. Protozoa often release nitrogen that can be used by plants while feeding on bacteria or other protozoa. Protozoa are most abundant and active near plant roots.

Soil Chemistry

Much of the chemical activity in a soil is associated with humus and clay particles. Clay is a natural mineral constituent of the parent material while humus is derived from decomposed organic matter. Both types of particles are characterized by an extremely large surface area to weight ratio and the presence of electrical charges on their surfaces to which ions and water molecules are attracted. Humus and clay exist in the colloidal state, a suspension of fine particles in water. On a weight basis, humus colloids hold more water and nutrients compared with clay colloids, but there is usually much more clay in a soil compared with humus. Thus clay often has more of an impact on nutrient and water holding capacities of a soil than humus.

The two major types of clay found in most soils are: (i) montmorillonite; and (ii) kaolinite. Montmorillonitic clay is less weathered than kaolinitic clay. Montmorillonite particles are composed of three layers, two of silica and one of alumina (the crystalline form of aluminum). These sheets are not held together very tightly, and as such tend to expand or contract as they become wet or dry. Soils high in this type of clay tend to be difficult to manage, often being very sticky when wet, and hard and cracked when dry. Montmorillonite soils have a higher cation exchange and water holding capacity compared with kaolinite soils. Kaolinite clay is highly weathered and is composed of two layers, one of silica and one of alumina. The two layers are held together more tightly than montmorillonite and therefore do not expand or contract as much when wetted or dried. They are also easier to manage.

Cation exchange capacity (CEC)

Clay and humus particles are negatively charged. With large surface areas, they both have a tremendous number of negative electrical sites on each particle, each site capable of attracting and holding particles with a positive charge (i.e. cations). These cations may be held to the soil particle surface or exchanged with another cation into the soil solution, hence the term CEC ('cation exchange capacity'). Once in the soil solution, the cation may be absorbed by a plant or it may combine with a negatively charged ion and leach out of the soil.

The CEC of a soil is important for understanding its nutrient holding capability. Soils with a high percentage of clay or organic matter have a high CEC and therefore can adsorb and retain applied fertilizers easily. Sandy soils have a much lower CEC and therefore cannot adsorb applied nutrients as easily and are subject to leaching. How tightly a cation is held to a soil particle is related to the CEC of the particle as well as the nature of the cation. Smaller cations or cations that contain less water tend to be held more tightly than larger or drier cations. Divalent cations are held more tightly than monovalent cations.

The CEC of a soil is expressed in milliequivalents (mEq); 1 mEq is 1 mg of hydrogen or the amount of any other cation that will displace it. Sand may have a CEC as low as 2 while clay may have a value as high as 150. Soil milliequivalents are expressed on the basis of a 100 g of dried soil. One milliequivalent is equal to 10 parts hydrogen per 1 million parts soil, or about 22 kg hydrogen/ha soil, 17 cm deep (20 lbs/acre, 6.67 in deep). This standard allows easy conversion of milliequivalents of other elements to kilograms per hectare, kg/ha (pounds per acre, lbs/acre). To determine kilograms per hectare (pounds per acre) for any other element, first determine the element's equivalent weight then multiply by 22.4 (20). The equivalent weight is equal to the element's atomic weight divided by its valence. For example, calcium has an atomic weight of 40 and a valence of 2, thus its equivalent weight is 40/2 or 20. Thus 1 mEq of calcium is equal to 20 × 22.4 or 448 kg/ha (20 × 20 = 400 lbs/acre).

Since laboratory estimation of a soil's CEC is time consuming and laborious, CEC is often estimated from soil test results for hydrogen, calcium, potassium, and magnesium. Divide the kilograms per hectare (or pounds per acre) for each component as reported on the soil test results by its corresponding milliequivalent weight (20 for hydrogen, 400 for calcium, 240 for magnesium, and 780 for potassium) then add the four values. This is the estimate of the soil's CEC. For example, suppose the test results for were H = 48, Ca = 780, Mg = 115, and K = 247. The estimated CEC for that soil would be [(48/20) + (780/400) + (115/240) + (247/780)] = 5.14.

Percentage base saturation

The percentage base saturation gives a reasonable idea of soil pH and fertility. The higher the percentage base saturation, the higher the pH and fertility. The percentage base saturation is calculated as the proportion of the total CEC accounted for by calcium, magnesium, and potassium. In our example, the base saturation would be (1.9 + 0.5 + 0.3)/5.14 = 53%.

Ions that are absorbed by plants from the soil solution are quickly replaced by a similar ion, if available, from the ions adsorbed to soil or humus particles. The capacity of a soil to replenish ions, particularly cations, from the soil solids to the soil solution is often called a soil's buffering capacity. This is different than a soil's pH buffering capacity, which is the soil's capacity to resist changes in pH. Soils with a high CEC have the potential to have a high buffering capacity if they are well fertilized.

Anion adsorption

Anion adsorption isn't as important as cation adsorption from a horticultural standpoint. Anion adsorption is regulated by soil pH and is only significant at a pH lower than that which would support plant growth. Common soil anions include chloride, phosphate, nitrate, and sulfate. Phosphates and sulfates are adsorbed more strongly than chlorides and nitrates. Occasionally adsorption of phosphate ions may be significant enough to cause concerns regarding availability for plant use. Most anions are lost from the soil by leaching.

pH

Soil pH greatly affects nutrient availability in the soil and is therefore of concern to horticulturists. Soils with a low pH are called acidic soils while those with a high pH are called basic or alkaline soils. But what does this mean? Recall from basic chemistry that an acid is a substance that releases hydrogen (H^+) ions while a base is a substance that releases hydroxyl (OH^-) ions. The strength of the acid or base is its tendency to release either hydrogen or hydroxyl ions when dissolved in water. Thus substances in soil water can contribute acidic or basic properties to the soil making the soil itself either acid, alkaline, or neutral.

A reasonable generality is that the more hydrogen ions held by a soil in relation to the number of basic ions (Ca^{2+}, Mg^{2+}, K^+) the more acidic the soil. Soils become more acidic as basic ions are replaced from the soil particles by hydrogen ions due to absorption by plants or leaching from the soil. Soils formed in area with high annual rainfall tend to have acidic soils. In addition, rain helps acidify soils. Carbon dioxide in the air combines with rainwater to form a weak acid, carbonic acid. In the soil solution, carbonic acid ionizes into hydrogen ions and bicarbonate ions, and the hydrogen ions replace calcium ions held by soil particles. The released calcium ions combine with the bicarbonate ions forming calcium bicarbonate, which is soluble in water and leaches out of the soil. Many fertilizers, especially those fertilizers that release ammonium, contribute to soil acidity. As ammonium is converted to nitrate via nitrification, hydrogen ions are released, acidifying the soil. Certain crops such as soybeans (*Glycine max*), alfalfa (*Medicago sativa*), and clover (*Trifolium* spp.) absorb more cations compared with anions while concomitantly releasing hydrogen ions to maintain an electrochemical balance within their roots, acidifying the soil.

Soil acidity is measured by its pH, a measure of the concentration of hydrogen ions in solution. Values near 1 indicate excessive acidity while values near 14 indicate excessive alkalinity. A value of 7 is neutral. The pH scale is logarithmic, meaning that a pH of 5 is ten times more acidic than a pH of 6 and 100 times as acidic as a pH of 7. Most crops grow best in a slightly acidic soil with a pH ranging from about 5.8 to 6.5. Of course there are exceptions, such as blueberry (*Vaccinium* spp.) and azalea (*Rhododendron*) which grow best at a soil pH near 5.0–5.5 and alfalfa (*M. sativa*), clover (*Trifolium* spp.) or sugarbeet (*Beta vulgaris*) that grow best in soils with a pH of 6.5–7.0. Another measurement called the buffer pH is an important indicator of the potential acidity of a soil. It is often included in a soil test report as BpH. The BpH measurement is used in calculating the amount of lime needed to increase the pH of the soil, if necessary.

Liming to correct pH

Many horticultural soils need liming to increase their pH. Raising the soil pH makes essential nutrients available for plant use and also prevents elements such as aluminum and manganese from reaching toxic levels. In addition, liming adds calcium (and magnesium if the lime is dolomitic) to the soil.

Materials used for liming contain calcium and/or magnesium in specific forms that neutralize soil acidity. The main differences among the materials available for liming are the costs of the material and the rapidity in which they raise pH. The faster acting materials cost more. Just because a material contains calcium or magnesium does not mean that it will change the soil pH. Gypsum (calcium sulfate) contains significant calcium, yet does not alter soil pH. When calcium sulfate is added to water, calcium hydroxide and sulfuric acid are produced and these two substances neutralize each other, thus there is no change in soil pH. When calcium carbonate (calcitic limestone) is added to the soil, it dissolves to form a weak acid (carbonic acid) and a strong base (calcium hydroxide). The hydroxide ionizes to calcium and hydroxyl ions and the cations replace hydrogen ions on the soil particle to neutralize the soil acidity.

Ground limestone is either calcitic (calcium carbonate) or dolomitic (calcium carbonate and magnesium carbonate). In some areas, limestone must contain at least 6% magnesium to be labeled 'dolomitic'. The effectiveness of either type of limestone in raising the soil pH depends on its purity and fineness of grinding. Hydrated lime, also called slaked or builder's lime, is calcium hydroxide. It is quick acting, but powdery and somewhat hard to handle. It is also more effective in neutralizing pH than ground limestone since it only takes 75% as much hydrated lime as ground limestone to neutralize the same soil situation.

The quality of any liming material is determined by its purity and particle size. Purity is expressed as calcium carbonate equivalents (CCE) or the neutralizing capacity of the material as compared to pure calcium carbonate. A high CCE is useless if the particle size is too large. Thus the two components must be considered together. Sometimes limestone is pelletized with clay to improve ease of handling, and when it is used, should be allowed to react with rain before incorporating into the soil.

When applying lime, sufficient time must be provided for the material to work prior to establishing a crop. This will depend on the form of liming material, its fineness, and the crop. A ballpark figure of 3–6 months prior to planting should allow sufficient time for the pH increase to occur. Even lime applied just prior to planting can be beneficial for raising extremely low pHs if the lime is finely ground and well incorporated. Liming material should be well incorporated into the topsoil after application if possible. With perennial crops or no-till scenarios, incorporation is not possible, thus it is even more important to use the most finely ground materials available and to apply it on a regular basis.

Excessive alkalinity

Some soils are naturally alkaline. Other soils are made alkaline by irrigation water that may be high in calcium or magnesium carbonate. Similarly, irrigation water may be high in sodium, further exacerbating the problem. Elemental sulfur is used to reduce soil pH in these situations. When the sulfur is applied to the soil, it combines with water in the presence of specific microorganisms to form sulfuric acid. This process may take from 3 to 6 weeks, depending on weather, soil conditions, and how fine the sulfur is ground.

Aluminum sulfate should not be used to lower soil pH. It contains aluminum, which at low pH (which is the direction you are aiming at with its application) may be in sufficient quantities to be toxic.

Soil salinity

Soil salinity and the problems it causes were covered in Chapter 6 of this volume.

Soil Testing

In order to make intelligent decisions concerning soil management, growers must know some basic attributes of their soil. This is what a soil test provides: a snapshot of the general condition of a soil including, but not limited to, its texture, sodium content, pH, and fertility levels. There are many commercial laboratories that specialize in soil testing including many labs at agricultural universities, and the tests they routinely perform vary. Before embarking on a soil testing venture, make sure you have evaluated your needs so that the appropriate tests are performed. You don't want to skip an important test and you don't need to pay for tests that you don't need.

Soil sampling

A good soil test begins with a good sample that represents the soil in question. If several soil types are involved in the management program, separate

samples should be taken and separate tests should be performed for each soil. The soil test is meant to estimate the entire soil, thus a good sample is crucial for effectively interpreting results and implementing recommendations. Remember, less than a tablespoon full of soil is used in the test which might represent a field of many hectares. Most growers now rely on site specific management which involves precise regulation of soil management of 2–3 acre parcels of land, rather than entire fields. Thus a field of 30 acres may now be managed as ten 3 acre sites rather than a 30 acre field. Site size depends on variability within the field. Greater variability requires smaller sites.

Soil samples are taken to estimate how well a particular site will support a specific crop, not to measure the nutrients in a particular parcel of land. Samples should be drawn with this goal in mind while taking into consideration the variability present in the field. Think of the sample of soil you send to the lab as a set of subsamples gathered from each field being tested. Labs will provide a specific set of sampling criteria that should be followed concerning collection of subsamples. Subsamples are normally drawn from a depth of 17 cm. Shallow samples will over-estimate the fertility of the field while samples deeper than recommended will probably underestimate cropping capacity of the soil. Samples should also be taken in an organized and documented pattern, taking care not to sample directly where banded fertilizer applications may have been made.

Stainless steel or chrome-plated sampling tubes or augers should be used and subsamples transferred to clean plastic buckets for mixing. Avoid galvanized or rubber buckets. Samples should be taken around the same time each year but at least 30 days after liming, sulfur, or fertilizer application. Always remove any surface debris before sampling and sample at a consistent recommended depth. Each sample should be made up of 10–15 subsamples, thoroughly mixed in a plastic bucket. Follow the lab's specific instructions for packaging and shipping your samples, making sure you have included the appropriate paperwork and fees.

Calibrating and interpreting soil test results

Soil test results must be calibrated to the crop they are intended to support. Calibration studies are performed by professionals over many years on many soil types. The testing lab normally performs the calibration for you, and this is why they request cropping information on forms submitted with your samples (Fig. 10.2). It is best to look at recommendations as suggestions for optimizing production. Improved crop performance may occur if recommendations are followed but there is no guarantee. The best yield responses are obtained when soil tests reveal a soil that is 'far away' from optimum and recommendations are followed closely. But remember soil fertility is only one part of the production equation.

Soil Fertility

Even though plants are autotrophs that synthesize their own food through photosynthesis, they have a requirement for obtaining certain nutrients, mostly ions, from an external source such as the soil or growing medium in the case of hydroponics (Fig. 10.3).

Plants require 17 essential mineral elements which are divided into macronutrients and micronutrients based on quantities needed for normal growth and development. The macronutrients include carbon, hydrogen, oxygen, nitrogen, potassium, phosphorus, calcium, magnesium, and sulfur. The micronutrients include iron, chlorine, copper, manganese, zinc, nickel, molybdenum, and boron.

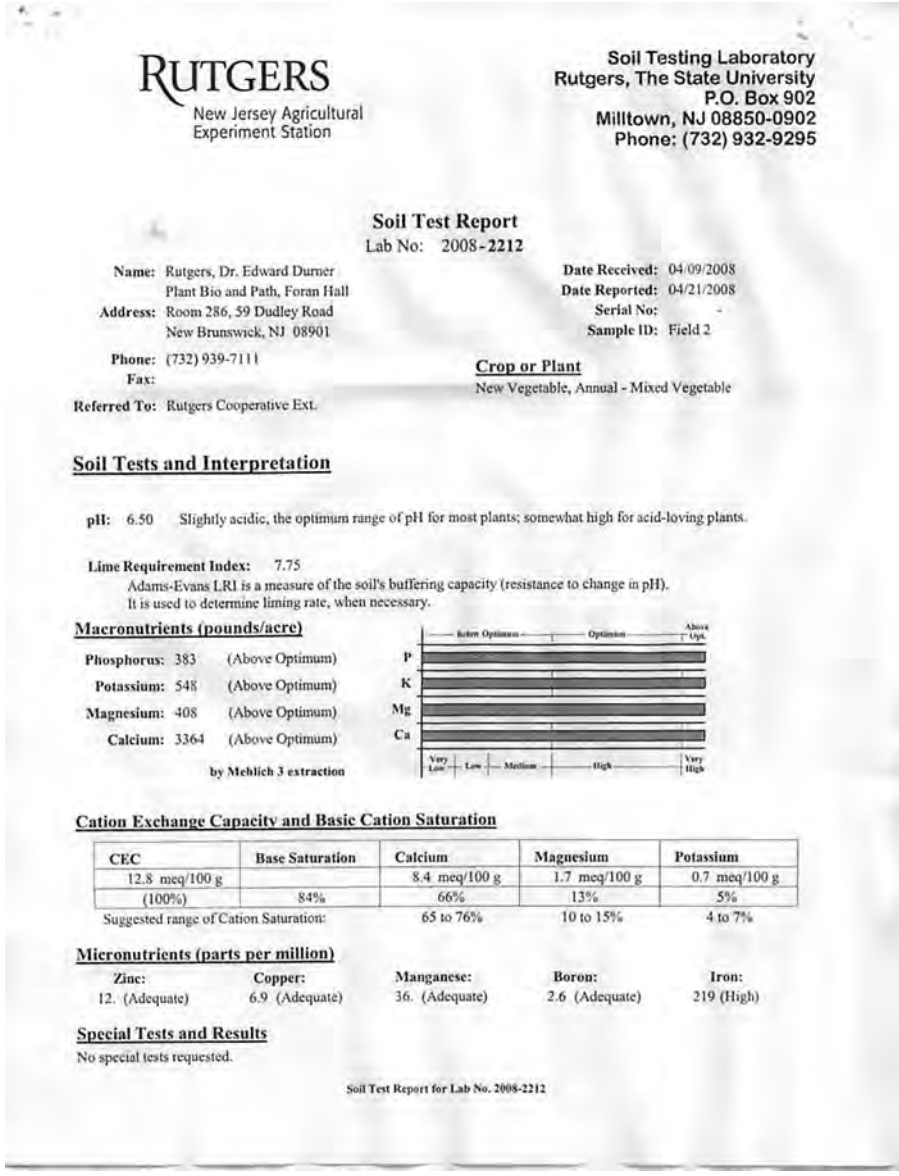
Macronutrients are often categorized as either primary or secondary. The primary nutrients include nitrogen (N), phosphorus (P), and potassium (K). They are called primary because they are usually lacking from the soil because plants use such large amounts of them. Soils almost always require supplemental N, P, and K for crop production. The secondary nutrients are calcium (Ca), magnesium (Mg), and sulfur (S). They are called secondary because one or more of them are often present and available in sufficient quantities such that fertilization with them is not required.

In this section, the major roles each nutrient plays in plant growth and development will be explored along with fertilizer sources for each nutrient, as well as a brief description of excess and deficiency symptoms.

Macronutrients

Carbon, hydrogen, and oxygen

Carbon, hydrogen, and oxygen together make up about 98% of a plant's fresh weight. These



RUTGERS
New Jersey Agricultural Experiment Station

Soil Testing Laboratory
Rutgers, The State University
P.O. Box 902
Milltown, NJ 08850-0902
Phone: (732) 932-9295

Soil Test Report
Lab No: 2008-**2212**

Name: Rutgers, Dr. Edward Durner
Plant Bio and Path, Foran Hall
Address: Room 286, 59 Dudley Road
New Brunswick, NJ 08901
Phone: (732) 939-7111
Fax:
Referred To: Rutgers Cooperative Ext.

Date Received: 04/09/2008
Date Reported: 04/21/2008
Serial No: -
Sample ID: Field 2

Crop or Plant
New Vegetable, Annual - Mixed Vegetable

Soil Tests and Interpretation

pH: 6.50 Slightly acidic, the optimum range of pH for most plants; somewhat high for acid-loving plants.

Lime Requirement Index: 7.75
Adams-Evans LRI is a measure of the soil's buffering capacity (resistance to change in pH).
It is used to determine liming rate, when necessary.

Macronutrients (pounds/acre)

Phosphorus: 383 (Above Optimum)
Potassium: 548 (Above Optimum)
Magnesium: 408 (Above Optimum)
Calcium: 3364 (Above Optimum)
by Mehlich 3 extraction

Cation Exchange Capacity and Basic Cation Saturation

CEC	Base Saturation	Calcium	Magnesium	Potassium
12.8 meq/100 g		8.4 meq/100 g	1.7 meq/100 g	0.7 meq/100 g
(100%)	84%	66%	13%	5%
Suggested range of Cation Saturation:		65 to 76%	10 to 15%	4 to 7%

Micronutrients (parts per million)

Zinc:	Copper:	Manganese:	Boron:	Iron:
12. (Adequate)	6.9 (Adequate)	36. (Adequate)	2.6 (Adequate)	219 (High)

Special Tests and Results
No special tests requested.

Soil Test Report for Lab No. 2008-2212

Fig. 10.2. Soil test results for a field used for mixed organic vegetable production.

elements are obtained from the atmosphere and soil as carbon dioxide and water. They are major components in many molecules involved in plant growth but since they are obtained from non-mineral sources, they are not normally covered in discussions of mineral nutrients. They were included here for completeness in our listing of plant essential elements.

Nitrogen

After carbon, hydrogen and oxygen, nitrogen is the most abundant element in plant tissues. In some species it can account for up to 4% of a plant's fresh weight. Even though plants are surrounded by an atmosphere that is 78% nitrogen, it is inert nitrogen gas (N_2) that is not directly available to

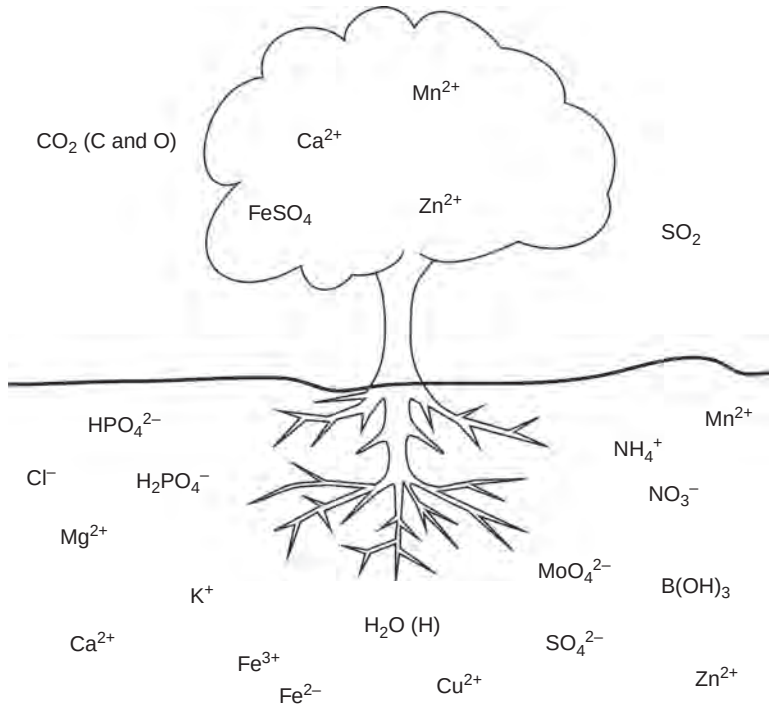


Fig. 10.3. Forms of nutrients absorbed by plants.

plants for growth and development. Nitrogen deficiency is the most common plant nutrient deficiency worldwide. With insufficient nitrogen, plants are weak, spindly, and unproductive.

Nitrogen is a key element of proteins which include the thousands of enzymes that facilitate life. Proteins are also key structural components in plant tissue. It is a major component of chlorophyll, and under deficiency conditions, chlorophyll production is reduced and leaves take on a chlorotic, yellow color. Nitrogen is a mobile element, meaning that once incorporated into plant tissues it can be remobilized if necessary and move to actively growing tissue. Since it is mobile, older leaves first show signs of nitrogen deficiency. Nitrogen is part of the ATP molecule, important in the metabolic energy balance of plants, and is also a part of nucleic acids, the genetic code for life.

Nitrogen in the soil exists as nitrogen in organic compounds, ammonium (NH_4^+) and nitrate (NO_3^-). Most soil nitrogen (95–99%) is present as nitrogen in organic matter such as plant and animal residue or microbes such as bacteria. This nitrogen is not available to plants, but a small portion of it can be converted to usable forms by microbes. A very small fraction of organic soil nitrogen may also be in a soluble form such as urea, which may be available for plant use. Most soil nitrogen available to plants is in an inorganic form, either NH_4^+ or NO_3^-. Since NH_4^+ is a cation, it binds to negatively charged soil particles such as clay or humus. On the other hand, NO_3^- is an anion and remains in the soil solution or precipitates out as a soluble salt under dry conditions. Being soluble, NO_3^- is easily leached from the soil and at times is leached at rates high enough to cause concerns about groundwater pollution.

Plants can absorb nitrogen as either NH_4^+ or NO_3^-, however, since most of the soluble inorganic nitrogen in a soil is in the NO_3^- form, most nitrogen is taken up as NO_3^-. Once absorbed, NO_3^- is converted to NH_4^+ then assimilated into biomolecules.

Nitrogen in the soil comes from two major sources: (i) fertilizer; and (ii) naturally occurring nitrogen. Naturally occurring nitrogen comes from either the nitrogen in minerals of the soil or the atmosphere. Very small quantities of nitrogen are slowly released as mineral and organic matter components of the soil breakdown. Most of the naturally occurring nitrogen in the soil comes from

the atmosphere through either of two mechanisms: (i) conversion of nitrogen (N_2) to nitrate (NO_3^-) by lightning in thunderstorms; or (ii) fixation by microbes in the soil. Nitrate produced during a thunderstorm dissolves in rainwater and is deposited in the soil. This process normally accounts for only 22 kg N/ha and the exact amount depends on the number of thunderstorms occurring in any year.

BIOLOGICAL NITROGEN FIXATION Nitrogen fixation by microorganisms is a much more important source of nitrogen for plant growth. This process called biological nitrogen fixation is accomplished by free-living organisms in the soil or by organisms living symbiotically with certain species such as legumes. Organisms that fix nitrogen utilize atmospheric nitrogen to synthesize nitrogenous compounds for their own growth and development. When they die and decompose, the nitrogen is released into the soil for use by other organisms, including plants. Since biological nitrogen fixation requires substantial amounts of energy, free-living nitrogen-fixing organisms such as *Azotobacter* fix minimal amounts of nitrogen each year because food sources for the energy required for fixation are often scarce. Symbiotic organisms such as strains of *Rhizobium* fix significantly more nitrogen. Rhizobia are bacteria that infect the roots of certain plants, mainly legumes, and form small nodules with a complex anatomy and biochemistry that allows them to live in symbiosis with the host plant (Fig. 10.4). The bacteria obtain carbohydrates from the host plant and, when the bacteria fix more nitrogen than they need, it is passed onto the host plant. In addition, when the host plant dies, nitrogen from dying Rhizobia is released to the soil for use by a future crop. Most of the fixed nitrogen is actually removed in the harvested crop. In fact, the field residue of legume plants remaining after harvest usually contains no more nitrogen than non-leguminous residue. Plants may relinquish 20–30% of their carbohydrates to *Rhizobium* over the growing season in exchange for as much as 225 kg or more N/ha/year.

The symbiotic relationship for nitrogen fixation is a complex one. *Rhizobium* bacteria invade cortical cells in legume roots where they quickly begin to multiply. The infection site begins to swell, forming the classic nodule seen on many legume roots. Young nodules do not fix nitrogen and are usually white or gray inside. Older nodules that are fixing nitrogen are pink or reddish colored. The reddish color is caused by leghemoglobin, a compound that regulates oxygen movement to the bacteria in the nodule. Nodules on perennial legumes such as alfalfa (*M. sativa*) are often fingerlike in shape and often look like a hand – a central mass with protruding fingers. These nodules are long lived and may fix nitrogen for the entire growing season. There are generally 10–50 nodules per plant, centered on the tap root. Nodules on annual legumes such as peas (*Pisum* spp.), beans (*Phaseolus* spp.), or peanuts (*Arachis hypogaea*) are short lived and about the size of a pea. They are constantly being replaced throughout the growing season. When pods of the host plant begin to fill, nitrogen fixation ceases, since carbohydrates are diverted from the nodules to the filling pods. Beans generally have less than 100 nodules per plant, soybeans may have several hundred, and peanuts may have well over 1000 nodules per plant.

Fig. 10.4. Nodules of *Rhizobium* on roots of green bean (*Phaseolus vulgaris*).

Healthy nodules will be pink or red during the growing season, indicating good nitrogen fixation. If nodules are white or gray, it means that they are not fixing nitrogen. Lack of nitrogen fixation may be due to: (i) a poor *Rhizobium* strain; (ii) poor

plant nutrition, especially phosphorus, potassium, zinc, iron, molybedenum, or cobalt; (iii) pod filling; or (iv) plant stress. Stress could include insects, disease, heat, or drought. If the stress is alleviated, productivity can often be improved and nitrogen fixation enhanced.

Some legumes fix nitrogen more effectively than others. Common beans (*Phaseolus* spp.) are poor nitrogen fixers, fixing less than 56 kg N/ha/year. Maximum productivity often requires a fertilizer supplement of 33–56 kg N/year. If beans are not well nodulated, productivity may remain low even with supplemental fertilization. The nodules somehow help the plants utilize nitrogen more efficiently. Peanuts (*A. hypogaea*), cowpeas (*Vigna* spp.), soybeans (*G. max*), and fava beans (*Vicia faba*) are good nitrogen fixers, fixing up to 280 kg N/ha/year. Maximum productivity with these crops requires no supplemental nitrogen fertilization, though a very small amount of nitrogen (11–16 kg/ha) may be supplied to seedlings prior to the onset of fixation. Too much supplemental nitrogen at this stage will inhibit fixation. Perennial and forage legumes such as alfalfa (*M. sativa*), clover (*Trifolium* spp.), and vetch (*Vicia* spp.) may fix as much as 280–560 kg N/ha/year and are not normally fertilized with supplementary nitrogen. Immediately after cutting, a small amount of nitrogen may be applied to supplement the lack of fixation while the plant re-establishes a photosynthesizing surface.

Adequate nitrogen fixation depends on an adequate supply of an appropriate and efficient *Rhizobium* strain. While *Rhizobium* is present in most soils, it may not be present in sufficient quantities or present as the appropriate strain for the crop being grown. Since inoculation is inexpensive ($1–5/ha), it is often a good idea to inoculate seed with an appropriate strain prior to planting. This is particularly true if: (i) the land has never been in cultivation; (ii) the legume you are planting has never been grown in the intended field; or (iii) it hasn't been grown there for more than 5 years. If the legume is not new, but you don't know how well-nodulated previous crops have been, it is wise to inoculate. There is no need to inoculate if the legume was recently grown on the same land and nodulation and nitrogen fixation appeared adequate. Once established, *Rhizobium* will persist in the soil for several years, thus there is no advantage to yearly inoculation of legume crops in fields where the well-nodulated legume is routinely grown. Once a particular strain of *Rhizobium* is established, it is hard to get another strain to establish itself if you decide to change crops. This is because the established strain is usually more competitive for the nodulation sites on the roots.

Inoculants are available as powders, granules, liquids, and on pre-inoculated seeds. Powders are the most common and reliable form of inoculant. They are black or tan, contain 1 billion *Rhizobium*/g and are stuck on the seed before planting. Granular inoculants are two to three times as expensive as powders and contain the same concentration of bacteria as powders. The larger particle size allows it to flow through an insecticide or fertilizer applicator and no mixing with seed is required. Granules are useful for pesticide-coated seed or seed with crops with a fragile seedcoat (e.g. peanut, *A. hypogaea*) which would be easily damaged by mixing. Liquids are easier to handle than powders, but be especially careful regarding expiration date and handling, especially temperature, as improper handling can kill the bacteria in the inoculant. Pre-inoculated seed is only worth the extra cost if seed are freshly inoculated and quickly planted. *Rhizobium* won't survive long when exposed to air, heat, cold, or light.

Inoculants must be handled correctly to be effective. *Rhizobium* is living and very sensitive to heat, desiccation, and light. Many inoculant failures can be traced to improper handling. They should be stored in a cool, dry place away from heat. Some pesticide and fertilizer coatings can kill the bacteria if the inoculant is applied directly to the seed. Check the inoculant's label for: (i) the type of legume it is intended for; (ii) the quantity of seed or acreage the inoculant will cover; and (iii) its expiration date. The inoculant must match the crop. If conditions are particularly hot or dry, double the recommended amount of inoculum. Never buy inoculant that has been stored in a fertilizer or pesticide warehouse. When inoculating the crop, follow label instructions, don't leave inoculants in the hot sun, and store leftovers appropriately. Use a sticker with powders, either a commercial one or a homemade one consisting of 266 ml sugar to 946 ml water. Don't use too much sticker and be as gentle as possible with seeds. Plant inoculated seeds immediately and don't expose treated seed to sunlight.

THE NITROGEN CYCLE The complex transformations of nitrogen in the soil are collectively called the nitrogen cycle. Most of the transformations are

carried out by microorganisms, thus they occur at very slow rates in cool or cold soils. As soil temperature increases, transformation rates also increase. The main focus of the nitrogen cycle is the interconversion of organic and inorganic nitrogen in the soil. As microorganisms grow, they absorb NH_4^+ and NO_3^- from the soil's inorganic nitrogen pool and incorporate it into their cells, becoming part of the organic nitrogen pool. This process is called immobilization. When these organisms die and decompose, NH_4^+ is released back into the inorganic nitrogen pool. This process is called mineralization. Mineralization also occurs when an organism decomposes organic matter in the soil and releases excess NH_4^+ into the inorganic soil nitrogen pool. How much inorganic nitrogen is available from the soil for plant uptake often depends on the balance between mineralization and immobilization that is occurring. Ammonium ions (NH_4^+) that are not quickly immobilized or absorbed by plant roots are quickly converted into NO_3^- in a two-step process called nitrification. In the first step, the bacteria *Nitrosomonas* converts NH_4^+ to nitrite (NO_2^-). Another bacteria, *Nitrobacter*, then converts the NO_2^- to NO_3^-. These two conversions usually occur at a rapid rate, thus much of the nitrogen in the inorganic soil pool is in the NO_3^- form. Nitrate is an anion and is easily leached from the soil. If soils become waterlogged and the oxygen supply in the soil is reduced, some bacteria convert NO_3^- to nitrous oxides and N_2, releasing oxygen for their respiration. The nitrous oxides and N_2 are not available for plant use and both are volatile and easily lost from the soil. This process is called denitrification. Significant losses of soil nitrogen can occur due to denitrification if soils are warm and remain saturated for more than a few days. Soils may also lose limited amounts of nitrogen through the volatilization of ammonia (NH_3), which readily forms from NH_4^+, particularly in alkaline soils. Nitrogen is also removed from the cycle in harvested crops.

Commercial nitrogen fertilizers

ORGANIC SOURCES Organic sources of nitrogen such as cover crops, compost, blood meal, feather meal, alfalfa meal, etc., rely on mineralization to release their nitrogen for plant use. A cover crop of alfalfa or clover can provide up to 224 kg N/ha/year, and about half of it will be mineralized during decomposition immediately following incorporation (Sarrantonio, 1994). This might be enough to supply the entire amount of nitrogen needed by the crop. However, cover crops require a long-term land commitment. For growers who cannot commit enough land to cover cropping and/or need to supplement nitrogen from an organic source, the process can be a bit difficult. The main problems with most organic sources of nitrogen is that they: (i) are expensive ($1–50/kg N); (ii) have a low nitrogen content; and (iii) the nitrogen in them is very slowly available to the plant.

Compost or manure can be added as a source of nitrogen. It is important to understand the principles behind using composts and manures for fertilization. Both manures and composts also supply other nutrients and they must be considered in decision making. Many types of composts and manures are available for field application. It is important to know: (i) what type of compost or manure you are using; (ii) if it is fresh or aged; and (iii) its nutrient content. Average nutrient contents for various composts and manures are available in any good reference, including Rosen and Bierman (2005). If possible, it is a good idea to have a sample of the product you are going to use tested. In addition, many products may not be derived from organic sources.

Composted manure is better to use than fresh manure for several reasons. Fresh manure is high in soluble nitrogen and salt build up, or losses from leaching, could occur with over-application. Fresh manure also may contain many viable weed seeds and may also contain pathogens such as *Escherichia coli*. If fresh manure is used, it should be applied at least 4 months prior to harvest. High temperatures generated in composting will kill most weed seed and pathogens. On the negative side, compost is usually more expensive than fresh manure and may reduce the amount of available nitrogen. In fact, nearly all of the nitrogen in compost is in the organic form which is not available to plants for uptake until it is mineralized by microorgansims in the soil. In addition, all of the nitrogen in compost may not be available to plants even after mineralization due to denitrification, volatilization, and leaching. Finally, only a part of the total nitrogen in compost is available to the plants in the year of application.

In general, 45–75% of the organic nitrogen in fresh manure is available the year of application while only to 14–30% is available from compost. Fresh manure should be incorporated immediately

into the soil after application to avoid losses from volatilization of ammonia. The C:N ratio of the manure or compost should also be considered as a C:N ratio of 25:1 or greater will greatly reduce the nitrogen immediately available to plants.

Unlike most conventional nitrogen fertilizers, organic sources such as compost and manure provide a residual supply of nutrients that must be accounted for in subsequent years of application. A general rule of thumb is that in the second and third years following application, 50 and 25%, respectively, of the organic nitrogen mineralized during the first cropping season will be available to the crop. Some manures and composts are especially high in phosphorus which can build to excessive levels. Routine soil and plant tissue tests should be performed to monitor nutrient levels over time.

In any fertilizer management scheme, whether organic or conventional, the first step is to determine crop needs. This information is readily available from reference books. For example, a general figure for the nitrogen requirement of mixed vegetables in the North-east USA is 125 lbs N/acre (140 kg/ha) (Maynard and Hochmuth, 1997). This figure will vary slightly with specific crop, soil and climate conditions, but it is a good reference point. The next step is to figure out how much nitrogen will be supplied by decomposing organic matter (not from the cover crop) in the soil. The organic matter content is often reported in soil test results. A rule of thumb is that 11 kg N/ha are released for every 1% organic matter, up to 4% (Grubinger, 2011). Assuming a soil with 3% organic matter, 33 kg N/ha would be available for plant growth; 107 kg N/ha are still needed, and we'll assume that a cover crop was not incorporated (not a very good organic practice!).

To determine the amount of compost or manure needed, the nutrient content of the material should be determined with testing or estimated from a table such as the one in Rosen and Bierman (2005). Most often since nitrogen is the nutrient most deficient, application rates are based on nitrogen needs rather than phosphorous (P) or potassium (K). The available nitrogen from your source is calculated as:

$$\text{Available N} = [(\text{Total N} - \text{Ammonium N}) \times (\text{Fraction of organic N released}) + \text{Ammonium N}]$$

Where total N and ammonium N are from a test result or estimated from a reference table and fraction of N released is from a reference table.

As an example suppose we are using fresh dairy manure with bedding which will be incorporated immediately after application. It has an estimated that the total N content is 4.5 kg/t and the ammonium N is 2.5 kg/t with the fraction of N released is 25%.

$$\text{Available N} = [(4.5 - 2.5) \times (0.25) + 2.5]$$

$$\text{Available N} = 3 \text{ kg/t}$$

In our example we still needed 107 kg N/ha, which means we need 107/3 or approximately 36 t/ha.

You should next determine the amount of P and K supplied by the manure to establish whether or not supplemental fertilizers are required for either. The P_2O_5 and K_2O content of the manure is obtained via test results or estimated and availability is generally 80% for P_2O_5 and 90% for K_2O.

For our example, estimated P_2O_5 content is 2 kg/t and K_2O is 5 kg/t, but only 80% and 90%, respectively, is available:

$$\text{Available P} = (2 \times 0.8) = 1.6 \text{ kg/t}$$

$$\text{Available K} = (5 \times 0.9) = 4.5 \text{ kg/t}$$

If we are applying 36 t/ha to satisfy the nitrogen requirements, we are applying 57.6 kg P_2O_5/ha and 162 kg K_2O/ha. Whether or not these amounts are sufficient depends on soil test results and recommended application rates for the two nutrients.

The same general procedure can be followed for calculating application rates for other organic nutrient sources. Other organic sources of nitrogen include but are not limited to: legume hay, grass hay, alfalfa meal, soybean meal, cottonseed meal, crab meal, fish meal, and feather meal.

CONVENTIONAL SOURCES Most commercial nitrogen fertilizers for conventional agriculture are manufactured from ammonia. Anhydrous ammonia (NH_3) is 82% nitrogen and is a gas at room temperature. It is stored and transported as a liquid under pressure that is injected several inches below the soil surface where it dissolves in water and forms ammonium (NH_4^+). Ammonia is manufactured using the Haber process, developed in the early 1900s. Gaseous H_2 and N_2 are combined under high temperature and pressure to produce ammonia. Manufacturing ammonia is an energy-intensive process not only for the production of ammonia itself, but for the production of the H_2 and N_2 that are used in the process.

Ammonium nitrate (NH_4NO_3) contains approximately 34% nitrogen. It is usually used as a solid and may be applied directly or in combination with other materials to create a custom-blended fertilizer. When ammonia is oxidized in air, nitric acid is produced. If the nitric acid is neutralized with more ammonia, a solution of ammonium nitrate (83%) in water (17%) is produced, which is then concentrated and sprayed into air forming small crystals of ammonium nitrate called prills. The prills are sized and sorted for sale.

Urea, $CO(NH_2)_2$, contains 46% nitrogen and is used as a solid or liquid. It is the most important solid nitrogen fertilizer worldwide (Eckert, 2011). When urea is applied to the soil, it is converted to ammonium carbonate by an enzyme called urease. Ammonium carbonate is unstable and breaks down into carbon dioxide and ammonia. If the ammonia does not dissolve in the soil water, it quickly volatilizes resulting in significant nitrogen losses to the atmosphere. This is a major problem with using urea as a nitrogen source, particularly in warm and humid climates. Mixtures of urea and ammonium nitrate are often dissolved in water to produce solutions with 19–32% nitrogen. The solution is then injected or applied as a banded side dressing, as overplant applications can cause severe tissue burning. Urea is manufactured from ammonia and carbon dioxide under heat and pressure.

Ammonium phosphates are manufactured from ammonia and phosphoric acid into many different formulations which include mono-ammonium phosphate (MAP), diammonium phosphate (DAP), and a few ammonium polyphosphates. They are mostly used as phosphorus fertilizers with the added benefit of supplying nitrogen.

A number of nitrate salts are available as nitrogen fertilizers. They include sodium nitrate (NaNO3, 16% nitrogen), calcium nitrate (Ca(NO3)2, 15.5% nitrogen), and potassium nitrate (KNO3, 14% nitrogen). They are expensive and contain relatively low amounts of nitrogen, thus other sources are often used.

Many slow-release forms of nitrogen are available and are particularly well suited to container production and turfgrass management. These products release nitrogen at low levels, slowly over time at a predictable rate. Compounds such as magnesium ammonium phosphate, ureaformaldehyde and isobutylidene diurea have very low nitrogen solubility in water. In addition the ureaformaldehyde and isobutylidene diurea rely on microbial degradation for nitrogen release. Other compounds, urea for example, can be coated with a polymer, wax or sulfur thereby slowing the release of nitrogen into the soil.

Phosphorus

Phosphorus is an important element in nucleic acids, particularly in the chemical bonds associated with them. Phosphorus is also important in energy metabolism: it is part of the ATP molecule. The bonds between phosphorus atoms hold the energy in ATP so vital for all metabolic processes. Cell membranes also contain a significant amount of phosphorus in the form of phospholipids. At the whole plant level, phosphorus stimulates: (i) root development; (ii) increased stem strength; (iii) improved flowering; (iv) better seed production; (v) uniform and early crop production; (vi) increased disease resistance; and (vii) more efficient nitrogen fixation in leguminous plants (Griffith, 2011).

After nitrogen, phosphorus is the nutrient most limiting plant growth (Schachtman *et al*., 1998). Phosphorus deficiency can often be difficult to detect and by the time a deficiency is detected, it is too late to remedy the problem with a fertilizer application. One symptom that often appears is a darkened, purplish-red coloration of leaves due to an accumulation of carbohydrates that enhances anthocyanin production. Phosphorus is mobile, thus deficiency symptoms appear in older leaves first.

Soil phosphorus content depends on: (i) parent material; (ii) degree of weathering; (iii) climate; (iv) previous crop production; and (v) fertilization. Organic forms of phosphorus come from manure, plant residue, compost, and microorganisms. From 20 to 80% of the phosphorus in soil is organic in the form of phytic acid (inositol hexaphosphate) (Richardson, 1994). Inorganic phosphorus occurs in the soil in over 170 forms (Holford, 1997). Some of the major forms of soil phosphorus are apatite, the source of all phosphorus, iron, and aluminum phosphates. Phosphorus is not very soluble in the soil and the average agricultural hectare contains less than 1 kg of available, soluble phosphorus. As phosphorus is removed from the soil by plants, it is quickly replaced (Griffith, 2011). Plants with extensive root systems are often more efficient at obtaining phosphorus from the soil (Lynch, 1995). Mycorrhizae are extremely important in plant phosphorus uptake because their hyphae effectively increase the surface area of the root system

(Smith and Read, 1997). Adding soluble phosphorus to the soil does not increase the amount of phosphorus available to plants as it is quickly fixed into insoluble forms. More than 80% of the phosphorus in a soil is unavailable to plants (Holford, 1997). Phosphorus is not very soluble and moves very little in the soil, around 10^{-12}–10^{-15}/m^2/s (Schachtman *et al*., 1998). It is not prone to leaching. Losses of phosphorus from the soil occur by crop removal and erosion.

In acidic soils phosphorus precipitates as iron and aluminum phosphates while in alkaline soils, it precipitates as slightly soluble calcium phosphate. Plants absorb $H_2PO_4^-$ ions more readily than HPO_4^{2-} ions and the former are favored by a soil pH between 6.0 and 7.0. When supplementary phosphorus and nitrogen are both needed, more phosphorus is absorbed by plants when applied as ammonium phosphate compared with other forms. High organic matter favors phosphorus availability to plants. The organic matter can chelate iron ions and make them unavailable for forming insoluble iron phosphates. Soils high in clay fix large amounts of phosphorus. To maximize the phosphorus available to plants, phosphorus-containing fertilizers should be applied just before it is needed by the crop. In addition, banding is recommended compared with broadcast applications. Cool, wet soils decrease oxygen in the soil which reduces the capacity of plants to absorb phosphorus.

Most responses to phosphorus application occur when soil tests indicate a low phosphorus level in the soil. With the increase in conservation tillage worldwide, response to phosphorus application even when soil tests indicate high soil phosphorus have been reported (Griffith, 2011). The reason for this 'new' response to phosphorus application is that conservation tillage results in lower soil temperatures and higher soil moisture levels especially at planting. These two factors often render phosphorus unavailable to plants, thus supplemental phosphorus enhances crop performance.

Given that phosphorus concentrations in the cytoplasm may be 1000 times greater than soil concentrations of phosphorus, plant root cells have specialized active-transport-uptake mechanisms for phosphorus. Energy is required to move phosphorus against a concentration gradient and against the negative electrical membrane potential of the cell membrane. Phosphorus is co-transported with one or more protons. Cells can import phosphorus in either of two forms, $H_2PO_4^-$ or HPO_4^{2-}, but phosphorus is mostly absorbed in the monovalent form (Ullrich-Eberius *et al*., 1984; Furihata *et al*., 1992). Once in the cell, phosphorus concentration in the cytoplasm is fairly constant while the vacuolar concentration varies considerably depending on soil phosphorus availability. When soil supplies are limited, the vacuole serves as a phosphorus reserve for the cell. Within the plant, phosphorus moves in the xylem primarily as inorganic phosphorus, and in the phloem as organic phosphorus. Movement in the phloem is usually a remobilization of phosphorus from older leaves to younger leaves during times of phosphorus deficiency.

While plant roots absorb phosphorus directly from the soil, in more than 90% of land plants, a symbiotic association between plant roots and mycorrhizal fungi exists (Smith and Read, 1997). There are two forms of mycorrhizae, ectomycorrhizae and endomycorrhizae, with vesicular arbuscular mycorrhizae the most widespread form in the plant kingdom. Vesicular arbuscular mycorrhizae are fungi that penetrate cortical cells of the root and eventually form structures called vesicles. Plants supply the fungus with carbohydrates while the fungus supplies the plant with phosphorus. Increased phosphorus uptake by mycorrhizal plants may be due to increased surface area of the effective root system due to fungal hyphae extensions into the soil and the ability of mycorrhizal fungi to more effectively scavenge for phosphorus in the soil compared with other soil organisms. Mycorrhizae may also be able to absorb organic forms of phosphorus that plants cannot from the soil. When soils are adequate in phosphorus, the degree of mycorrhizal colonization of roots is often limited. Specific signals from the plant rather than phosphorus concentration in the soil appear to regulate the degree of colonization (Tawaraya *et al*., 1996).

SOURCES OF PHOSPHORUS Rock phosphate is mined from the earth and finely ground for application to the soil. It has very little phosphorus available for plant use, thus it is not used as a fertilizer very often (Rehm *et al*., 2010). Rather, it is used to manufacture phosphoric acid, an ingredient for other manufactured forms of phosphorus. DAP, $(NH_4)_2HPO_4$, which is 18% nitrogen and 46% P_2O_5, is manufactured by reacting ammonia with phosphoric acid. When applied to the soil, it causes a temporary increase in pH as one molecule of ammonia is released. As the product continues to

break down, it has an acidifying effect on the soil. Another product of reacting ammonia with phosphoric acid is MAP, $NH_4H_2PO_4$. It contains 11% nitrogen and 52% P_2O_5. Bone meal, 22% P_2O_5, is a slow-release organic source of phosphorus.

Potassium

Plants absorb potassium as a cation, K^+. Potassium is not a structural part of plant tissues nor is it an element in metabolic compounds. Rather, it is an element which is essential for regulation of many processes within the plant. It is crucial in regulating: (i) photosynthesis; (ii) photosynthate translocation; (iii) protein synthesis; (iv) opening and closing of stomata; and (v) the activation of at least 60 enzymes (Thompson, 2011).

Potassium is mobile therefore deficiency symptoms appear in older tissue first. A common symptom of potassium deficiency is severe chlorosis of leaf margins which may be followed by premature defoliation of the plant.

While potassium comprises about 2.4% of the earth's crust, from 90 to 98% of the total potassium in the soil is not available to plants (Thompson, 2011). Only about 0.1–2% of the total potassium in the soil is in the available form, K^+. When soil K^+ is high, plants may suffer a magnesium deficiency and vice versa.

Potassium is mined from sylvinite, sylvite, and langbeinite. While it does not exist in the plant or soil in this form, potassium content is expressed as a percentage K_2O. Sylvinite is mostly potassium chloride (KCl) and sodium chloride (NaCl), containing 20–30% K_2O. Sylvite is mostly potassium chloride with 60–62% K_2O. Langbeinite is mostly potassium sulfate (K_2SO_4) and magnesium sulfate ($MgSO_4$) containing 22% K_2O, 11% magnesium, and 22% sulfur.

Potassium chloride is also called muriate of potash and accounts for most of the potassium used in fertilizers. Potassium sulfate, also called sulfate of potash, is often used as a potassium source in chloride-sensitive crops such as tobacco (*Nicotiana* spp.), fruit crops, and some vegetables (Thompson, 2011). It accounts for about 2–6% of the potassium used in fertilizers. Mined langbeinite is often called double sulfate of potash and is a good source of potassium, magnesium, and sulfur. Potassium nitrate (KNO_3) supplies both nitrogen (13%) and potassium (44% K_2O).

Sulfur

Sulfur is absorbed as the sulfate anion (SO_4^{2-}) by plant roots and may be absorbed to a lesser degree as sulfur dioxide (SO_2) by leaves. Sulfur is an important component of cysteine and methionine, two sulfur-containing amino acids. It is also important for efficient nitrogen fixation by legumes and for conversion of nitrates to amino acids. The pungent flavor of *Brassica* and *Allium* species is due to sulfur-containing compounds. The very sweet and mild onions such as 'Vidalia' or 'Maui Sweets' are grown on soils with very low sulfur content to minimize the production of pungent sulfur compounds.

Most of the sulfur in soil is tied up in organic matter and is not available for plant use until it is converted into the sulfate (SO_4^{2-}) by bacteria in a process similar to mineralization of nitrogen. Sulfate is soluble and negatively charged, thus it tends to be leached from the soil much like nitrate. Sulfates can move upwards in the soil via capillary action as water is drawn to the surface with evaporation. Soil tests and crop calibrations for sulfur are difficult due to this mobility.

Sulfur deficiency is often confused with nitrogen deficiency since both are first noticed as chlorosis of leaves with a general stunting of growth. Sulfur is immobile, thus as symptom intensity increases, chlorosis appears on younger leaves; nitrogen symptoms occur on older leaves. Since soil tests for sulfur are unreliable, plant tissue analysis provides a better measure of deficiency. Tissue concentration of sulfur should be between 0.2 and 0.5 %. The total nitrogen to total sulfur ratio should lie between 7:1 and 15:1. Values greater than 15:1 may indicate a sulfur deficiency.

If irrigation water contains 5 ppm or more of sulfur, sulfur deficiency is highly unlikely. If elemental sulfur is applied to improve sulfur fertility, it must be converted to sulfate by bacteria before the plant can utilize it. This can take from 3 to 6 weeks, depending on moisture and temperature conditions in the soil. Other sulfur-containing fertilizers can also be used, most of them with sulfur in the form of sulfates. Ammonium sulfate, $(NH_4)_2SO_4$, contains 21% nitrogen and 24% sulfur. It is a good source of both sulfur and nitrogen, however, it does significantly lower the soil pH. Approximately 15 kg of lime must be used per kilogram of nitrogen applied to neutralize the acid formed. Gypsum, or calcium sulfate, $CaSO_4$, is a ready source of calcium and sulfur with the added benefit that it

does not alter the soil pH. Sulfur-coated urea has 11% sulfur and is a good source of slow-release nitrogen.

Calcium

Calcium is absorbed by plants as the cation Ca^{2+}. It is important in cell wall structure and also an important messenger ion in the plant.

In the soil Ca^{2+} is adsorbed to soil particles and released into solution. If soils are properly limed, calcium deficiency should not be a problem for most crops. However, a number of horticulturally important crops suffer from calcium-related problems. Blossom end rot in tomatoes (*Solanum lycopersicum*) is due to insufficient calcium transport to young fruit, even when sufficient levels of calcium are present in the soil. Similarly cork spot and bitter pit of apple (*Malus domestica*) is due to insufficient calcium in the fruit, not the soil. When deficiencies do occur, they are observed in younger tissues as calcium is immobile in the plant. A classic symptom is marginal necrosis of young leaves.

The best source of calcium is calcitic limestone, provided during good soil pH management. If magnesium is also deficient, dolomitic limestone is a good choice, providing both magnesium and calcium while increasing the pH. If calcium is needed and there is no need to increase the pH, calcium sulfate (gypsum) is a good choice.

Magnesium

Magnesium is absorbed as a cation, Mg^{2+}, and is essential for photosynthesis. The center of each chlorophyll molecule is a magnesium atom. Magnesium is also important in phosphorus translocation, cell division, protein formation, and is an important activator of enzymes.

Magnesium availability to plants is dependent on soil pH. At a low soil pH (<5.8), magnesium availability decreases due to interactions with hydrogen ions and aluminum ions. At higher pH levels (>7.4), interference from calcium may reduce magnesium uptake by plants. If soil test readings are below 25–50 ppm, supplemental magnesium is warranted. A rule of thumb based on the CEC of a soil test is with a CEC higher than 5 mEq/100 g, it is desirable to maintain a soil Ca:Mg ratio of about 10:1 (Snyder and Thompson, 2011).

Magnesium is mobile and deficiency symptoms appear in older leaves first. Interveinal chlorosis begins near the tips or margins of the leaves and progressively moves inwards towards the central axis of the leaf. Leaves often become brittle and curl upwards. Tips and margins may turn reddish purple with a severe deficiency. In general plants require about 0.2% tissue magnesium. Some crops that require significantly more magnesium than usual include forage legumes and grasses, cotton (*Gossypium* spp.), oil palm (*Elaeis* spp.), corn (*Zea mays*), potatoes (*Solanum tuberosum*), citrus (*Citrus* spp.), sugarbeet (*B. vulgaris*), soybeans (*Glycine max*), celery (*Apium graveolens*), and tobacco (*Nicotiana* spp.).

A popular source of magnesium is dolomitic lime, containing 6–12% magnesium depending on source. It contains magnesium as magnesium carbonate which is quite insoluble in water so it is only slowly available for plant uptake. More finely ground lime makes more of the magnesium and calcium available more quickly than less finely ground product. When dolomitic lime is used, another form of more readily available magnesium should be used as well.

Sulfate of magnesium potash, also known as K-Mag and also a good source of potassium and sulfur, is 10–11% water-soluble magnesium which is readily available to crops. It is one of the most economical forms of magnesium available (Snyder and Thompson, 2011). Magnesium sulfate, more commonly known as Epsom salts, contains about 10% soluble magnesium and can be used as a foliar spray. It is quite expensive. Commercially available sources of less refined magnesium sulfate contain 16–18% magnesium, but it is not completely water soluble so it cannot be used as a foliar spray. It is useful as a soil application.

Micronutrients

Iron

Iron is absorbed by plants as the cations Fe^{3+} or Fe^{2+}, and is most available from slightly acidic soils. It is important in chlorophyll synthesis, thus a deficiency is easily recognized as chlorosis in younger leaves. If the deficiency is severe enough, leaves will turn nearly white before browning and dying. Iron deficiencies are exacerbated by high levels of zinc, phosphorus, and manganese in the soil. Iron is also important for the activity of many enzymes, especially those involved in energy transfer, nitrogen reduction and fixation, and lignin formation.

Iron is most effectively supplemented with foliar applications of a 3% solution of $FeSO_4$ at 187–374 l/ha (Mortvedt, 2011).

Boron

Boron is absorbed by plants as borate, $B(OH)_3$, and is important in cell wall formation, formation of vascular tissue, carbohydrate metabolism, flower formation, and in pollen formation and germination. Boron is immobile in the plant so deficiencies appear at the growing point, causing extreme rosette growth. Other symptoms of boron deficiency include poor fruit set due to poor pollination, hollow stems and fruits, brittle discolored leaves, and flower bud abscission.

Boron deficiencies are often found on acidic, sandy soils in regions with high rainfall and or low organic matter. Borate ions are soil mobile, thus leaching can occur. Recommended rates of application for boron are very low, from 1 to 2 kg/ha. Extreme care should be given to accurate application since the difference between adequate and toxic levels is very small.

Copper

Copper is absorbed by plants as Cu^{2+}. It is important in carbohydrate and nitrogen metabolism as well as lignin formation. Deficiency symptoms include stem and twig dieback, stunted growth, and chlorotic or pale green leaves leaves that easily wilt. Copper absorption decreases with increasing pH and phosphorus and iron in soils may inhibit copper uptake. Copper is often supplemented at the rate of 3–12 kg/ha of copper as copper sulfate or finely ground copper oxide and its effects may last for up to 8 years (Mortvedt, 2011). Careful monitoring of soil and plant tissue copper levels are needed to prevent copper toxicity.

Zinc

Zinc is absorbed by plants as Zn^{2+}. It is an essential cofactor for many enzyme systems, especially those involved in energy metabolism, protein synthesis and growth regulator activity. Zinc is plant immobile with deficiency symptoms appearing in young tissue. Symptoms include small leaves and a highly rosette shoot growth. Chlorotic banding in monocots and mottled leaves of some dicots are also characteristic symptoms of zinc deficiency. Delayed maturity is also caused by zinc deficiency.

Plant uptake of zinc decreases as soil pH increases and may be inhibited by high phosphorus and iron levels in the soil. Supplements of 1–12 kg zinc/ha are often recommended as foliar sprays or band applications of zinc sulfate. Zinc supplementation is residual for up to 5 years.

Molybdenum

Molybdenum is taken up by plants as MoO_4^{2-} and is mobile in the plant. It is involved in bacterial nitrogen fixation in legumes and is important in most enzyme systems. Both nitrogen and sulfur metabolism as well as pollen formation require molybdenum. Most plants never exhibit molybdenum deficiency. Legumes may exhibit molybdenum deficiency as a nitrogen deficiency, since the molybdenum deficiency is impacting nitrogen fixation by *Rhizobium*. In some vegetable crops, molybdenum deficiency causes irregular leaf blade formation called whiptail. Molybdenum uptake increases with increasing pH, thus liming to correct pH may correct a molybdenum deficiency.

Manganese

Manganese, absorbed as Mn^{2+}, is important in photosynthesis and nitrogen metabolism. Interveinal chlorosis is a classic symptom of manganese deficiency. Uptake decreases as pH increases and may be adversely affected by high iron in the soil. Annual banded applications of manganese as manganese sulfate or manganese oxide at 2–22 kg manganese/ha are often needed.

Chlorine

Chlorine is a non-mineral absorbed by plants as an anion, Cl^-. It is mobile within the plant and is important in the regulation of electrical charges throughout the plant and for stomatal functioning.

Chloride deficiencies in nature are extremely rare, but when deficient on sandy, wet soils, can be easily corrected with KCl.

Micronutrient fertilizers

Micronutrient supplements come in four forms: (i) inorganic products; (ii) synthetic chelates; (iii) natural organic complexes; or (iv) fritted glass

products. Inorganic sources are mostly oxides, carbonates or metallic salts such as nitrates, chlorides or sulfates. Synthetic chelates are compounds that effectively hold the micronutrient through covalent bonding and readily release it for plant uptake. Natural organic complexes are derived by reacting metallic salts with byproducts of the wood pulp industry forming a readily decomposed carrier for the micronutrient. Frits are small glass beads in which the micronutrients are embedded during manufacture. Their release depends on glass composition and frit size. Frits are normally used for maintenance of micronutrient levels rather than for correcting deficiencies.

Since application rates for micronutrient supplementation are normally very low, they are most often soil applied using granular or fluid NPK fertilizers as a carrier. The micronutrients can be incorporated during manufacturing, blended with NPK fertilizers to create a custom mix, coated onto granular fertilizers or mixed in with liquid fertilizers.

Foliar sprays are often used for correcting micronutrient deficiencies, especially iron and manganese. Application rates are much lower than for soil-applied amendments and uniform distribution is easily accomplished. Deficiency symptoms normally disappear within a few days of application if they were indeed due to a micronutrient deficiency, thus deficiencies can be corrected immediately during the growing season when first noticed.

Fertilizers and their use

Rates of application for any nutrient should be derived from either soil or tissue tests combined with specific crop requirements. Fertilizer application is an expensive and environmentally challenging endeavor, thus planning for applications is wise. Rates for nitrogen fertilization are probably the most difficult to determine as so many factors are involved in managing crop nitrogen. First, different yield objectives will lead to different nitrogen requirements. In addition, the amount of nitrogen required for application will depend on how much might be provided by the soil. This is governed by release of nitrogen from organic matter and crop residues as well as any residual nitrogen left from a previous crop. Previous cover cropping and compost or manure application must also be taken into consideration.

Fertilizers may be applied broadcast, banded with row crops, or applied foliarly. Placement and application should minimize potential injury to plants by fertilizer burning and maximize crop utilization of the nutrients applied. Broadcast fertilizers need to be incorporated into the soil while banded applications must be deep enough to prevent nitrogen losses, yet not so deep that the plant roots cannot absorb the nutrients.

If possible, fertilizers should be applied as close to the time of critical crop need as possible. For this reason, split applications, especially of nitrogen, are often made. However, split applications increase costs by requiring additional trips across the field. A good rule of thumb is to apply fertilizers within 30 days of planting or to actively growing crops.

Nitrate content of water supplies, particularly drinking water supplies, are of global concern. Ingestion of large amounts of nitrate by infants can lead to a potentially fatal condition called methemoglobinemia. This blood disorder leads to excessive production of methemoglobin, a form of hemoglobin that does not release the oxygen it is carrying to body tissues. Excessive fertilization with nitrate-containing fertilizers can potentially contaminate wells and groundwater.

Mulches

Mulches are an integral part of any horticultural production system. Mulches may be used year round depending on their purpose. The mulch may be as simple as plant debris on top of the soil, to applied compost or synthetic soil covers. No matter what form they take, mulches serve a host of common functions. All mulches: (i) conserve water; (ii) inhibit weed growth; (iii) prevent erosion; and (iv) influence soil temperature. The degree to which they accomplish these effects varies with mulching material. Organic mulches have the added benefit of adding organic matter to the soil as they decompose as well as providing nutrients. Living mulches such as white clover also provide nitrogen while contributing the other benefits of mulching. Winter mulches provide protection from widely fluctuating soil temperatures and plant desiccation by cold, dry winds.

Many different materials are used as mulches including, straw, compost, leaf mold, pine needles, newspapers, kraft paper, sawdust, biodegradable films made from starch, oxo-degradable mulches, landscape fabric, plastic mulch, and living mulches.

Organic mulches

Organic mulches are any material composed of organic matter used as a mulch in plant management. The term organic does not necessarily imply that the material is from an organically grown source. This is important for certified organic growers since many mulches composed of organic material are from conventional sources and may contain pesticides and fertilizers not approved in certified organic production.

Most organic mulches are environmentally friendly, renewable, biodegradable, and contribute to soil health with nutrients and humus. Organic mulches are also expensive. With any organic mulch, care must be exercised to ensure that enough supplemental nitrogen is supplied to the crop being mulched to compensate for nitrogen tied up in microorganisms that are breaking down the organic matter. Composted organic materials make better mulches than non-composted materials for this reason. Some composted mulches may tie up nitrogen as well, depending on the degree of decomposition of the compost material.

Biodegradable mulches

As an alternative to non-recycleable LDPE (low density polyethylene) plastic mulches, biopolymers made from polysaccharides and thermoplasticized, extruded starch films have been developed and used with varying degrees of success. These materials are biodegradeable in the soil by bacteria and fungi and are turned under after usually one crop (Moreno and Moreno, 2008).

An interesting alternative to biodegradeable films are biodegradeable materials such as sodium alginate, glucomannan, chitosan and cellulose that are sprayed directly onto the soil. When the water carrier of such materials evaporates, a thin mulch remains. The main drawback to these sprayed-on mulches is that they are still in the developmental stages so costs cannot be estimated and they often degrade too quickly once applied to the soil (Immirze *et al*., 2009).

Paper mulches, such as kraft paper and newspaper, are biodegradable. Ink type should be considered when recycling newspapers. Kraft paper may be impregnated with vegetable-oil-based resin made from soybean or linseed oil and citric acid (Shogren, 2000) which lengthens the time before degradation occurs and increases strength against weed penetration. Another advantage to impregnated paper is that they can be made from 100% recycled paper, natural or organic oils, and citric acid from corn sugar. It is reasonable to surmise that using brown kraft paper impregnated with polymerized oil would offer some degree of soil warming and moisture retention.

Oxo-degradeable mulches

Oxo-degradeable mulches are polyethylene-based plastic mulches with additives containing iron, nickel, cobalt, and manganese to cause accelerated degradation that is initiated by heat and light (Thomas *et al*., 2010). Manufacturers claim that the mulch will effectively disappear by the end of the growing season, thus eliminating the need for removal from the field. Oxidative degradation is induced by heat and light and results in the material fragmenting into very small pieces of mulch. These materials do not biodegrade quickly into harmless components (carbon dioxide and water) as often implied. The clear distinction between a material that undergoes oxidative degradation and one that is biodegradable is not often emphasized in marketing these products. Oxo-degradable plastics are not compostable.

Living mulches and cover crops

Cover crops and living mulches are two additional management tools for controlling weeds, erosion, and nutrient losses from cropland. A cover crop is any crop grown primarily to cover bare soil during times of the year when cash crops are not being grown. Cover crops are also those crops used to enrich soil with organic matter and nitrogen fixed by legumes during crop fallows or to rebuild soils depleted during normal crop production. Living mulches are species grown concurrently with the cash crop as a ground cover to provide all the benefits of a non-living mulch. Living mulches also act as a cover crop in that they return substantial amounts of organic matter and nutrients to the soil.

Winter cover crops are sown in late summer or early fall to grow through the winter or grow during the fall, go dormant during the winter months, and if hardy enough, resume growth in the spring. Before planting the spring crop, the cover is turned under or killed. A common winter cover crop is winter rye (*Secale cereale*). Winter hardy legumes such as alfalfa (*M. sativa*), hairy vetch (*Vicia villosa*), red clover (*Trifolium pratense*), white clover

(*Trifolium repens*), and sweet clover (*Melilotus* spp.) also make excellent cover crops, adding nitrogen to soils low in nitrogen. Some legumes may actually remove nitrogen from the soil if it is available, rather than fix it (Kaspar *et al*., 2008). Brassicas such as oilseed and forage radish and mustards make good winter covers that suppress nematodes, diseases, and some weeds (Kasper *et al*., 2008).

Living mulches are companion cover crops planted either before or with the main crop and left as a living cover during the growing season (Hughes and Sweet, 1979; Hartwig and Ammon, 2002). They are often perennial and remain in the field for many years or may be annuals or perennials that must be replanted regularly. The main problem with living mulches is that they tend to compete with the cash crop for water and nutrients. Living mulches have been used for years in vineyards and orchards and are being tested for adaptation to row crops. In orchards and vineyards, the living mulch is often some distance away from the vine or tree to reduce the competition for water and nutrients but maintain the benefits of a mulch in the row middles.

In order to be suitable as a living mulch, a species must possess a number of desirable attributes. For example it should be: (i) able to establish rapidly and have good wear tolerance; (ii) tolerant of drought and low fertility; and (iii) able to enhance crop yield and quality with minimal costs (William, 1987). Grasses and legumes are the primary species considered for living mulches. Suppression of the living mulch growth during production of the cash crop is the main difficulty with using a living mulch. Mulches are suppressed with reduced herbicide rates, mowing, rototilling, and animal grazing of living mulch sod middles in tree fruit orchards. Even with mulch suppression, the cash crop yield is often reduced compared with yield under other mulching or clean cultivation systems. In addition, living mulches often reduce soil temperatures and may harbor insect or disease pests (Paine and Harrison, 1993).

Landscape fabric mulches

Landscape fabric mulches, also called geotextiles, are typically manufactured from polypropylene or polyester, are long lasting but expensive and non-degradable. They are woven, perforated or spun-bonded sheets used extensively as an underlay for landscaping projects around trees and shrubs which utilize some form of mulch as an overlay. Pebbles or gravel are the best overlay as organic mulches degrade and form a layer of soil-like debris in which weeds can grow. Polyester fabrics last longer than those made from polypropylene.

Landscape fabrics are porous, thus air and water easily pass through to the soil, yet they prevent weeds from pushing through the fabric. They are usually pinned to the soil with plastic or metal fasteners and in general, will last up to 5 years. It is often easier to position fabrics around plants after planting rather than to try and plant through the fabric, especially with larger specimens.

Plastic mulches

Plastic mulches are very common, especially in row crop production of flowers, vegetables, and fruit. They are used to: (i) reduce weed growth; (ii) conserve moisture; (iii) reduce wind and water erosion; (iv) prevent leaching of valuable nutrients; and (v) diminish the development of plant diseases from soil-borne organisms. Plastic mulches also alter the soil and plant microclimate much more than other mulch types. In general, plastic mulches warm the soil leading to earlier maturing spring crops and extended harvests of fall crops. Plastic mulched crops are often harvested 7–14 days earlier than non-mulched soils, the product is often cleaner and yield is often two to three times higher, especially in sweet corn (*Z. mays*), eggplant (*Solanum melongena*), tomatoes (*Solanum lycopersicum*), muskmelons (*Cucumis melo*), peppers (*Capsicum annuum*), cucumbers (*Cucumis sativus*), summer squash (*Cucurbita* spp.), okra (*Abelmoschus esculentus*), and watermelon (*Citrullus lanatus*) (Lamont, 1993). When combined with drip irrigation, water savings of 45% can be realized using plastic mulch compared with bare soil. Most weeds will not grow under black plastic mulch. Nutgrass (*Cyperus rotundus*) is an exception: it easily punctures plastic due to the energy derived from food stores in its tubers (Fig. 10.5).

Linear low-density polyethylene black mulches are mostly used due to their: (i) high durability; (ii) resistance to chemicals; (iii) reasonable ease of use; and (iv) widespread availability (Lamont, 1993). Most plastic mulch is 1.25 mil thick (1 mil is one-thousanth of an inch, equivalent to 0.031 mm; it is a common description for plastic mulch thickness) and 48 inches (122 cm) wide and comes in rolls

Fig. 10.5. Nutgrass (*Cyperus rotundus*) puncturing black plastic mulch.

2400 ft (731 m) long. The plastic is usually smooth or embossed with a diamond-shaped pattern to reduce expansion and contraction which loosens the mulch from the soil, decreasing its effectiveness. Recently, colored mulches with different light-reflecting properties that alter plant growth, often enhance productivity and crop quality, and discourage insect pests have been introduced (Lamont, 1993). Plastic mulches also provide the opportunity to double or triple crop the land using only one application of mulch.

The negative side of plastic mulches is their environmental impact (Fig. 10.6). Most plastic mulch is not recycled, and unfortunately, some growers simply leave the plastic in the field and incorporate the material into the soil or burn it. New 'plastics' made from biopolymers of polysaccharides from corn (*Z. mays*), potato (*S. tuberosum*) or rice (*Oryza* spp.) are degraded by bacteria and fungi in the soil into carbon dioxide, methane, water, and biomass.

Decisions on which mulch to use are based on: (i) the crop; (ii) the season of production (spring, summer or fall); (iii) single or multiple cropping; and (iv) pest management considerations. The standard colors of mulch used today are black, clear, and white-on-black. Red, green, blue, and silver mulches are used to a lesser extent. They are crop specific and more expensive than the standard colors (Orzolek and Lamont, 2011).

The selection of black, white-on-black (henceforth white), or clear depends on how a grower wants to impact the temperature microclimate around the crop and the soil. Black mulch is an opaque black body absorber and radiator. This means it absorbs most UV, visible and infrared radiation and re-radiates the absorbed energy as thermal or long-wave infrared radiation. Most of the absorbed energy is lost to the atmosphere through radiation and convection. Good soil-to-mulch contact improves heat transfer from the mulch to the soil. Daytime soil temperatures are generally 2–3°C higher under black plastic mulch compared with bare soil.

Clear plastic mulch absorbs little radiation but transmits up to 95% of the incoming radiation, depending on thickness and opacity. In addition, the surface of the mulch next to the soil is usually covered in water droplets which are transparent to incoming short-wave radiation but opaque to outgoing long-wave infrared radiation. Thus much of the heat that would normally be lost to the atmosphere from a bare soil is trapped under the clear mulch. Daytime soil temperatures under clear mulch can be 3–8°C higher under clear plastic mulch, depending on the depth in the soil, compared with bare soil. The disadvantage of clear mulches is that they require fumigation, solarisation, or a fumigant to control weeds.

White or silver reflecting mulches result in a slight decrease in soil temperature of about 1 or 2°C. Most of the light intercepted by these mulches is reflected back into the plant canopy. Some white mulches require additional weed control measures. Some blue- and red-colored mulches can raise soil temperatures dramatically, as much as 18°C.

A group of mulches called photoselective mulches are available that transmit infrared radiation and absorb photosynthetically active radiation (PAR), which provides somewhat of a compromise between clear and black mulches in terms of soil temperatures. Photoselective mulches increase soil temperatures like clear mulches but do not have their weed problem. These mulches are usually blue- green or brown in color. They are more expensive than either black or clear mulch.

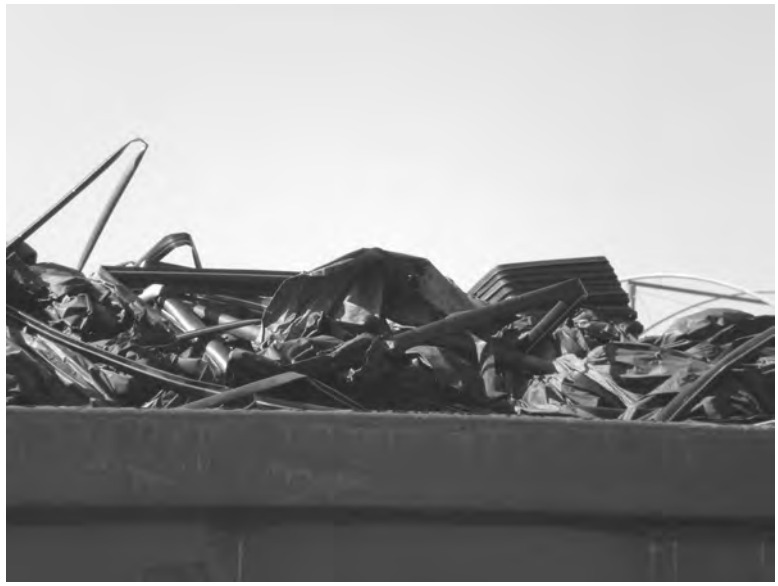


Fig. 10.6. Dumpster (large rubbish receptacle) containing non-recyclable agricultural plastics used in vegetable production. Waste includes plastic mulch, greenhouse plug trays, germination flats, and trickle tape.

Plant growth is also affected by phytochrome- or temperature-mediated foliar responses induced by mulch color. Clear mulch reflects nearly twice as much PAR than black mulch or bare soil, since the black mulch and soil absorb much of the PAR. The reflected PAR leads to larger plants with higher yields.

Red and black mulches similarly raise soil temperatures. Higher and earlier yields of certain plants, for example peppers (*C. annuum*) and tomatoes (*S. lycopersicum*), on plants of smaller stature are often observed with red mulches compared with black. Both mulches reflect similar amounts of PAR, but the red mulch reflects substantially more red than far-red light. More red light than far-red light tips the P_r:P_{fr} ratio in favor of P_{fr}, leading to smaller plants with higher yields. Other colors under investigation for their radiation-absorbing and reflecting properties include blue, green, yellow, orange, and gray.

Mulch color can also influence insect activity around the crop. Yellow, red, and blue mulches tend to attract certain insects while silver mulches repel them, especially aphids (Orzolek and Lamont, 2011).

Some general recommendations for mulch color selection for specific vegetable crops typically grown on plastic mulch were made by Orzolek and Lamont (2011). Keep in mind that results will vary, especially in locations significantly further south than Pennsylvania, USA, so be sure to consult local recommendations. When growing conditions were less than ideal, tomatoes (*S. lycopersicum*) produced significantly more fruit and exhibited fewer symptoms of early blight when grown on red mulch compared with black. When growing conditions were ideal, no differences were detected. Peppers (*C. annuum*) produced significantly more fruit when grown on silver mulch compared with black. Eggplant (*S. melongena*) responded with increased production to red mulch, especially when grown under stressful conditions. Muskmelon (*C. melo*) responded to green infrared-transmitting or dark blue mulch with a third more fruit than when grown with black mulch. Cucumber (*C. sativus*) and summer squash (*Cucurbita pepo*) responded to dark blue mulch with a 30% and 20% increase in marketable yield, respectively.

11 The Greenhouse Environment

A greenhouse provides the luxury of producing almost any crop at any time of the year regardless of the outside conditions, provided care is taken to establish and maintain the correct environment inside. Precise control of nearly all of the factors affecting plant growth and development is what makes a greenhouse the productive structure that it is. Greenhouse management encompasses many factors including temperature, light, moisture, carbon dioxide levels, growing media, fertility, and pest management. All of the factors that affect plant growth in the field also affect greenhouse production except for possibly wind. While all of the factors previously listed impact greenhouse production, this chapter will focus on how temperature, light, moisture, and carbon dioxide management can profoundly affect greenhouse productivity.

Temperature

Two different aspects of temperature must be considered in greenhouse operations: (i) heating; and (ii) cooling. Heat for a greenhouse comes from natural heat from solar radiation and supplemental heat supplied by a source chosen by the grower. While the choice for supplemental sources of heat is entirely under the control of the grower, solar heating is not. Estimates of solar heat input can be made based on daylength and sun angle, but other factors such as cloud cover and outside temperature cannot. Cooling is accomplished through ventilation and the use of some form of supplemental cooling, most often one that relies on the evaporation of water. The level of cooling by evaporation is subject to the water content of the air.

Heat sources

Heat in a greenhouse produces two areas of concern for growers. One is that there may not be enough heat during cold winter months and the other is too much heat during the summer. The first is remedied by supplemental heating while the other is addressed via ventilation and cooling.

Solar heating

When solar radiation strikes an object, say a polyethylene (poly) greenhouse covering, a plant or a greenhouse bench, that energy can be reflected, absorbed, or transmitted through the object. With the poly covering, most of the energy is transmitted. With the plant, some is transmitted, some is absorbed, and some is reflected. With the bench, most is absorbed or reflected. Most of the solar radiation entering a greenhouse is short-wave radiation. Much of that radiation which is absorbed by objects is converted to heat or re-emitted as long-wave, mostly infrared radiation. Water in the greenhouse atmosphere absorbs much of the re-emitted far-red radiation and converts it to heat. Thus much of the solar energy entering a greenhouse becomes heat. The fairly small amount of energy that is not converted to heat is used in photosynthesis.

Some of this heat absorbed during the day is stored and released at night. Systems for heating a greenhouse with stored solar heat have long been used in homeowner and small establishments. Large darkly colored containers filled with water collect and store solar heat during the day and radiate it into the greenhouse at night. This heating is passive and cannot be controlled thermostatically. It is inexpensive and easy to use.

Commercially available solar collectors and heat distributors are extremely expensive and would not be cost effective for economical greenhouse production at this time (Buffington *et al*., 2010). However, these systems will probably become more affordable in the future.

Supplemental heating

During cooler months some form of supplemental heat is needed for nearly all greenhouse production

schemes. Both traditional and not so traditional alternative heating systems can be used to supply this supplemental heat.

TRADITIONAL FUELS Oil, natural gas, and propane are traditional fuel sources for greenhouse heating. Fuel selection is based on burner type and economics (Bartok, 2005). Natural gas is piped from transmission lines to the greenhouse, thus no on-site storage is needed. It is generally economical, clean burning, and requires little equipment maintenance. Natural gas is sold by the therm, the volume of gas required to produce 100,000 British thermal units (BTU). One problem with natural gas is that some suppliers include an 'interruptible clause' in their sales contracts which allows them to divert gas to other customers in the event of severe cold weather. Thus an alternate heating source is needed as backup for the greenhouse.

Propane (liquefied petroleum gas) is a clean fuel like natural gas. It is liquid at moderate pressure and typical temperatures. Propane is normally stored on site in a large tank, thus it is available where natural gas is not. It is usually a little more expensive than natural gas, is sold by the gallon and contains about 95,000 BTU/gal (25,096 BTU/l).

Fuel oil No. 2 is about the same in cost as natural gas, except that oil burners require more maintenance than natural gas burners. It requires aboveground storage with a containment plan in case of a leak or spill.

Gas or propane burners often distribute the heat as forced hot air through poly tubes with holes punched in them. The tubes are mounted overhead and suitably distributed throughout the production area. The poly tubes can also be mounted under benches, keeping the heat in the plant zone. This type of heater is called a unit space heater (Fig. 11.1). The heater itself is mounted on the floor or hung from the greenhouse roof with the air distribution tubing appropriately installed to deliver the heated air to where it is needed. These units are moderately expensive and allow for easy greenhouse expansion if desired. It is extremely important to vent these units since combustion produces carbon dioxide, carbon monoxide, ethylene, sulfur dioxide, and unburned hydrocarbons. Many of these byproducts of combustion are potentially harmful to plants and humans if they are at elevated levels. High levels of carbon dioxide and water vapor that often develop at night when heaters are operating regularly are extremely conducive to growth of disease-causing organisms.

Fig. 11.1. A natural-gas-fueled greenhouse unit space heater. Notice the heater is well ventilated to the outside.

To ensure good heater ventilation, make sure the vent stack is appropriately sized for the heater (see manufacturer's recommendation) and at least 1.2 m higher than the highest point of the greenhouse (Buffington *et al.*, 2010). It is also important that ventilation to bring oxygen for combustion into the greenhouse is established, especially for tightly sealed greenhouses. Normally a vent 15–20 cm in diameter is located close to the heating unit to allow fresh air to be drawn in from the outside when the heater is on.

While space heaters are semi-permanent, many greenhouse furnace systems utilizing coal, oil, natural gas, or propane as fuel are permanent. They consist of a burner, a boiler, and a heat distribution system as well. The heat is normally distributed via either hot water or steam through a piping system appropriately configured for the greenhouse and the system. In general, hot water systems heat and cool more slowly and require more distribution piping than steam systems. Hot water systems are usually less expensive and simpler to install than

steam systems. Hot water systems are usually used in smaller greenhouse ranges while steam systems are used in larger ones. Steam systems can also be used to sterilize soil.

In both systems, heat is often distributed through a system of tubing or pipes either embedded in a porous concrete floor or mounted to production benches. Tubing mounted to production benches maintain production containers at a suitable temperature resulting in improved plant growth and reduced fuel costs. Fuel costs are reduced since production containers are heated directly through conduction rather than by radiation or convection with associated heat loss through the air.

Another type of oil- or gas-fired heater called a return stack or 'salamander' heater designed for orchard frost protection should never be used to heat a greenhouse. They are not vented and cannot be thermostatically controlled. With no venting, combustion gases would accumulate inside the greenhouse potentially injuring plants or sickening humans working near them.

Thermostats for controlling the heating system must be mounted appropriately in the production area for accurate sensing. Sensors should be mounted at plant level rather than eye level and sensors should be distributed throughout the production area. They should not be in full sun, but rather, facing northwards or in a ventilated and aspirated shelter.

ALTERNATIVE FUELS Alternative fuels are often used to supplement traditional greenhouse heating systems rather than for supplying the majority of supplemental heat (Bartok, 2005). They are usually units that are separate from the main system, thus they incur an additional expense. They also normally require more effort in operation and greater maintenance. Alternative sources of heat include biomass, coal, waste oil, methane, waste heat, heat pumps, and geothermal sources.

Biomass fuel is a generic term for any fuel derived from biological material including, but not limited to, wood, crop residue, and biomass byproducts from industry (Jenkins, 1985). Biomass can be used directly as a fuel or after gasification. It is relatively inexpensive but is also produces less heat per unit than other sources of fuel. Fuel storage must be considered and fuel must be kept dry and accessible to the heating system. A consistent and reliable supply is also necessary. Particulate emissions may be a problem with biomass fuels and ash must be disposed of properly. If properly managed, biomass furnaces can: (i) save fuel costs; (ii) pay for themselves within 4 years; and (iii) substantially reduce carbon dioxide emissions compared with conventional systems (Callahan and Grubinger, 2010). Wood is probably the most widely used biomass fuel and an alternative energy source for supplemental greenhouse heating. Wood as fuel is available as traditional firewood, forest residue, lumber or paper mill waste, chips, and sawdust. It is relatively inexpensive, but does require a night fireman to feed the furnace.

Coal may be a viable alternative energy source depending on location and availability. One ton of coal has the heat value of about 640 l (160 gal) of fuel oil or 2300 therms of natural gas. A coal furnace also needs a fireman and ashes must be disposed of properly.

Waste oil from automobiles may be used as an alternative fuel, but it must be cleaned to remove sludge and water before use. It is also considered a hazardous waste, thus it must be handled appropriately. It has a heat value similar to fuel oil No. 2.

Methane is a gas produced from landfills and decomposing animal manure. Methane that is burned directly from the source has the heat value of about 17,657 BTU/m^3, or half that of clean methane.

Many manufacturing plants generate waste heat in their processes. This heat can be used for greenhouse heating if the two enterprises are in close proximity to each other. Often the waste heat is lower grade, meaning that it is water or steam at 65°C or less, but even so, it is a valuable source of supplemental heat.

Geothermal heating of the greenhouse is an alternative to fossil-fuel-generated heat. Heat from the earth or bodies of water such as lakes, wells or ponds is captured by heat pumps and transferred to greenhouses. Heat pump/geothermal systems are usually quite expensive, require electricity as a power source and must be located near a convenient source of transferable heat. For these reasons, heat pumps are an alternative heating source for greenhouses in very limited situations (Jenkins, 1985; Garco *et al*., 1998).

Heat pumps use electricity to transfer heat from a source outside of the greenhouse to the interior growing area. Sources of the heat can be outside air, surface water, groundwater, soil, or solar radiation. The efficiency of the heat pump in extracting heat from the environment and transferring it to

the greenhouse is called the coefficient of performance (COP) which can range from 1 to 4. When the temperature of the environment is closer to the desired temperature of the greenhouse, the COP has a higher value. A heat pump extracting heat from groundwater at 8°C to heat a greenhouse maintained at 15°C has a higher COP than a heat pump extracting heat from air at 3°C. When the COP is near 1, a heat pump is no more efficient at heating than an electrical resistance heater. Thus heat pumps should only be considered when a heat source fairly close to the desired greenhouse temperature is available. In addition, electricity costs must be evaluated compared with other heating systems and their fuel sources.

Maintenance

To most effectively, efficiently and economically utilize greenhouse space, it is important to semi-annually check certain components of the greenhouse, its structure, and its heating and ventilation systems (Bucklin *et al*., 2002). This is particularly important for winter production. It's a good idea to schedule a thorough inspection of greenhouse facilities before each production cycle. Structural integrity of the greenhouse frame as well as the covering must be maintained. Repair or replace any component that is in disrepair. Fiberglass, glass and poly coverings should be checked for holes and for gaps between the covering and framework. Unnecessary shading should be removed and side panels should be cleaned to allow maximum light penetration. If possible, an inner layer of poly should be added to single layer coverings to insulate the greenhouse. Approximately 10 cm should separate the inner and outer coverings and the space between the two coverings should be maintained with inflation fans. Warm, dry air should be used for inflation to minimize condensation between the two layers.

Vents and ventilation fans should be checked for proper operation. If thermal blankets are used, they should also be checked for tears and to see that they operate properly. Since many greenhouse operations rely on a standby generator in case of a power outage, this too should be regularly inspected, tested, and serviced. The heating system should be inspected by a trained professional. Efficient and proper operation often relies on delicate adjustments best left to professionals. The cost of service is likely to be recouped via fuel savings with a properly adjusted system.

Calculating heat requirements

Appropriately sized supplemental heating systems must be used for efficient production. In order to select the correctly sized unit, an estimate of the heat loss from the greenhouse is required. Since many growers are incorporating high tunnels into their production scheme, let's determine the appropriate heating unit for supplemental heating that might be required on occasion for such a unit. Consider a 14.5 m (48 ft) long caterpillar high tunnel, 3.7 m (12 ft) wide and 2.1 m (7 ft) tall (Johnny's Selected Seeds, 2011).

A fairly accurate method for estimating heating requirements is easily obtained by multiplying the surface area of the high tunnel by the maximum temperature difference between the inside and outside air, then multiplying this number by a factor which adjusts for covering material and construction quality (Buffington *et al*., 2010). The adjustment factor for the high tunnel covered in polyethylene ranges from 1 for very tight construction to 1.5 for less than perfect construction. To be on the safe side, assume less than perfect construction.

The first step is to calculate the exposed surface area (A) of the high tunnel covering. A reasonable approximation to the surface area of the covering must consider the area of the two end walls plus the area of the tunnel. The tunnel arch consists of 6.1 m (20 ft) of appropriately bent pipe to produce the 2.1 m (7 ft) height at the center. Thus the area of the covering is $6.1 \times 14.5 = 88.5$ m^2 ($20 \times 48 = 960$ ft^2). Each end is approximately 0.5 times the surface area of a circle that has a radius of 2.1 m (7 ft), thus each end has a surface are of $0.5 \times (\pi r^2) = 0.5 \times (\pi \times 2.1^2) = 6.9$ m^2 (77 ft^2). Thus the total area (A) for the covering is (88.5 + 6.9 + 6.9 = 102.3 m^2) ((960 + 77 + 77) = 1114 ft^2).

Suppose the high tunnel is being used for an early tomato crop where we wish to maintain a minimum temperature of 15.5°C (60°F) (a cultivar tolerant of cooler temperatures is utilized). The minimum outside temperature anticipated is –9.5°C (15°F) (assuming an unexpected cold snap is better than not being prepared for one). The maximum temperature difference would be 15.5 – (–9.5) = 25°C (60 – 15 = 45°F). The approximate heating requirement for our high tunnel is $(102.3 \times 25 \times 1.5) \times 19.6 = 1114 \times 45 \times 1.5 = 75195$ BTU/h. The factor of 19.6 is required to adjust values derived from metric measurements. One BTU is the amount of energy it

takes to increase the temperature of 0.454 kg of water from 3.8°C to 4.4°C (or the energy needed to raise 1 lb of water 1°F from 39 to 40°F).

Once the heating requirement is determined, the selection of a heating system is primarily based on the economics of purchasing and operating the system. The least expensive, most reliable system should be chosen. Fuel type often determines operating costs. If possible, a system that allows conversion from one fuel type to another is desirable. Besides cost, reliable fuel availability should also be considered. If the greenhouse operation is located in a remote area which often experiences electrical power outages, an electrical system should not be considered even if it is the least expensive option. Heating efficiency and heat production for common greenhouse heater fuels is presented in Table 11.1. With these figures and local cost estimates, it is fairly easy to calculate costs associated with the various fuels based on heating requirements previously calculated.

In all but the simplest operations, an expert should always be consulted in order to determine the best heating system for a greenhouse. Careful planning virtually eliminates large problems down the road.

Ventilation

Ventilation is an extremely important component of greenhouse temperature and moisture management. It replaces warm humid air inside the greenhouse with cooler drier air from the outside. In addition, ventilation renews carbon dioxide levels in houses where carbon dioxide enrichment is not practiced. This is particularly important in smaller houses during the winter months. Ventilation may be forced or it may be accomplished naturally.

Table 11.1. Combustion efficiency and heat production for various fuels used in common greenhouse heating systems (after Buffington *et al.*, 2010).

Fuel	Efficiency (%)	Heat produced per unit (BTU)
Coal (bituminous)	60	13,000/lb
Wood	70	8,000/lb
Natural gas	80	100,000/therm
Propane	80	92,000/gal
Fuel oil No. 2	70	138,000/gal
Electricity	100	3,413/kwh

Natural ventilation

Natural ventilation is often accomplished by rolling greenhouse sidewalls up or down depending on configuration, allowing outside air to enter (Fig. 11.2). Ridge vents and open roof greenhouses accomplish the same thing with a different approach (Fig. 11.3). The degree of ventilation is regulated via the amount the side curtain is raised or lowered. While inexpensive and easy to operate, this type of ventilation is not easily regulated as much of the air exchange depends on wind. In addition, if curtains are manually regulated, someone must be available to open and close the curtains every day. Ideally, curtains should roll downwards to open. This allows colder air from outside to mix with the warmer greenhouse air before coming into contact with the plants. This is especially important when ventilating sensitive plants on cold, windy days. Greenhouse orientation should be such that the prevailing winds blow across the greenhouse rather than along its length.

Forced air ventilation

Forced air ventilation is accomplished by having a louvered vent at one end of the greenhouse and an exhaust fan at the other. Both are operated by a thermostat with the vent opening several degrees before the fan to prevent the development of a vacuum inside the greenhouse. Once the desired temperature is reached, the fan shuts off and the louvers close.

A good general rule of thumb for greenhouse production is that during ventilation, the air inside

Fig. 11.2. Natural ventilation of a high tunnel using roll-up sides.

Fig. 11.3. Natural ventilation of an open roof greenhouse.

the greenhouse should be exchanged with outside air once a minute. A fan of sufficient capacity must be used to ensure that the volume of air in the greenhouse can effectively be drawn out of the greenhouse in about 1 min. Fans are often capable at operating at two speeds, a lower speed for minimal ventilation and a high speed for maximum needs. Single-speed fans run the risk of bringing in too much cool air which might harm sensitive species during times of minimal ventilation.

Fans are most often selected based on how much air they can move in a specific amount of time, normally cubic feet per minute (CFM). The volume of air a fan can move depends on: (i) blade diameter; (ii) motor horsepower; and (iii) housing shape. Another important variable in calculating air flow is the system static pressure. As more air is moved, the resistance to flow increases, thus the amount of air that can be moved begins to decrease. A good rule to follow is to make sure you purchase a fan that can deliver the desired air flow at a minimum of 0.125 inches of static pressure. Fan manufacturers supply performance charts to indicate air movement of their fans at various static pressures. The louvers used in the ventilation system should be about 1.5 times larger than the fan frame to allow sufficient airflow.

Estimating the size of greenhouse exhaust fans is easily accomplished using the following formula (Snyder, 1992):

> Exhaust fan capacity needed (CFM) = 8 × (Length of greenhouse (ft) × Width of greenhouse (ft))

Estimating the size of greenhouse vents is easily accomplished using the formula (Snyder, 1992):

> Vent size (ft^2) = (8 × (Length of greenhouse × Width of greenhouse)/700

Greenhouse air movement

Part of a ventilation strategy should consider keeping air moving inside the greenhouse even at times when the outside air is not being vented in. This keeps temperature and humidity levels fairly even within the greenhouse and moving air helps eliminate condensation of water on plant leaves in cool pockets.

Air movement inside the greenhouse is often a component of the poly tube heat delivery system. Even when the heat is not needed or in cases where heat distribution is not accomplished via a poly tube, air circulation within the greenhouse could be accomplished with a poly tube. The poly tube system directs air down the center of the greenhouse via a high velocity fan through an overhead pressurized polyethylene tube with strategically spaced outlets. The tube runs the length of the greenhouse. A vent is often included as part of the system to allow outside air to be drawn in if needed for cooling purposes. The air exiting the tube via the outlets is moving at a high velocity and mixes the air around and within the plant canopy.

Another option is the horizontal air flow system which consists of 40–60 cm (16–24 inch) diameter fans positioned about 1.2 m (4 ft) above the crop

every 9–12 m (30–40 ft) along the length of the greenhouse. Fans are located about one-fourth the width of the greenhouse in from the exterior wall along both sides of the greenhouse. Fans are positioned in such a way as to move the air down one side of the greenhouse and up the other in a somewhat circular direction. Use high efficiency fans specifically designed for horizontal air movement. They should blow parallel to the ground, not pointed downwards or upwards as sometimes suggested (Runkle, 2012a). Fans should also be mounted solidly, not hanging from chains, as horizontal movement of fans can alter air movement within the greenhouse. If possible, fans should cycle off when ventilation is occurring. It is especially important to have fans operating at night to prevent pockets of cool air from developing throughout the greenhouse. Air movement at night also minimizes condensation on plants as they cool.

Cooling the greenhouse

Most greenhouses reach excessive temperatures during the summer growing season and are unfit for crop production unless cooled. Evaporative cooling is the most widely used method of greenhouse cooling (Bucklin *et al.*, 2011). Air conditioning can be used, but installation and operation costs are prohibitive.

To obtain a measure of potential cooling with evaporation, obtain the wet bulb temperature in the early afternoon. This is when maximum cooling will be required. With a well-managed and maintained system, the greenhouse temperature can be cooled to within 1 or 2°C of the wet bulb temperature.

Evaporative cooling of greenhouses is accomplished by evaporating water into an airstream usually with a fan-and-pad system. High pressure fogging systems can also be used, but are substantially more expensive.

The fan-and-pad system consists of an exhaust fan located at one end of the greenhouse which draws outside air through a vent at the other end of the greenhouse. As the air enters the greenhouse through the vent it passes through a porous pad, usually made of cellulose, through which water is trickled with a circulating pump. It is important to have a tightly sealed greenhouse so that air enters only through the pad to maximize evaporation. Water evaporates and cools the air entering the greenhouse. Each gallon of water evaporating removes 8100 BTU of heat from the air entering the greenhouse. Air is coolest immediately after passing through the pad and entering the greenhouse and warms slightly as it approaches the fan. The temperature gradient should be as small as possible. Air may warm by as much as 1°C every 6 m it travels. Additionally, the cooled air tends to travel in an angle up and away from the plants it was intended to cool. In a cross-greenhouse flow configuration, this is usually not a problem, as gutters connecting roof sections of large greenhouses provide baffles to deflect cool air downwards. In smaller greenhouses, the distance from side to side is short, thus the air divergence is not of great concern. In lengthwise-flow configurations, baffles should be created every 10 m and extend from the roof of the greenhouse down to just above crop level.

Estimating the size of greenhouse cooling pads is easily accomplished using the following formula (Snyder, 1992):

> Pad height (ft) = (Air flow rate)/(Pad length)/(Air velocity)

The air flow rate is the volume of the greenhouse since you want one air exchange per minute, thus air flow rate = greenhouse length × greenhouse width × 8. The pad length is usually about 2 feet shorter than the greenhouse width. Air velocity (in feet per minute) is how fast the air can move through the cooling pad. This number is obtained from the manufacturer and is approximately 250 for a 4-inch cellulose pad, 380 for a 6-inch cellulose pad, and 165 for an aspen pad.

If the efficiency of the cooling system is known, the temperature of the air exiting the cooling pad can be estimated as follows:

$$T_{cool} = T_{out} = (\text{Percentage efficiency})(T_{out} = T_{wb})$$

Where:
T_{cool} = temperature of cooled air
T_{out} = temperature of outside air
T_{wb} = wet bulb temperature of outside air

A well-designed system can have an efficiency of up to 85%. To illustrate how effective pad cooling systems can be, a system with 85% efficiency can take outside air at 32.2°C and a relative humidity (RH) of 50% down to 24.7°C. That's a 7.5°C decrease in temperature.

Greenhouse temperature and plant growth

When contemplating greenhouse temperature management it is wise to consider the many ways in which temperature influences plant growth and development. The rates of many plant processes are highly dependent on temperature. Within a range, warmer temperatures tend to increase while cooler temperatures tend to decrease the rates of metabolic reactions involved in these processes. Seed germination is highly regulated by soil temperature. Vegetative propagation is also affected by soil temperature. Photosynthesis, respiration, transpiration, and many other metabolic processes involved in synthesis and degradation are affected by temperature. Flower formation and production may be highly regulated by temperature in some species. Water and nutrient absorption are affected by temperature which ultimately may affect crop maturity.

Greenhouse temperature settings vary among species and are generally set to maximize yield. Night temperature is often emphasized for two reasons. One is that this is the time where the temperature normally has the greatest opportunity to fall below optimum levels. Secondly, much of a plant's growth occurs at night.

A species optimum temperature changes as a plant grows from a seedling to a mature plant. Growth only occurs if photosynthesis is greater than respiration, since a surplus from photosynthesis is needed for growth to occur. When a plant is young, it often has a great deal of leaf area (mostly photosynthesis) relative to stem and root area (mostly respiration). Warmer temperatures are usually favored by younger plants. Even though warmer temperatures cause increased rates of both photosynthesis and respiration, warmer temperatures favor photosynthesis and net growth in younger plants since there is less stem and root tissue compared with older plants. As the plant ages, more stem and root tissues develop, thus warmer temperatures start to favor respiration. Lower temperatures reduce photosynthesis but also reduce respiration, thus generally favoring a surplus of photosynthates that can be used for growth.

Plant growth in cooler greenhouses

In an attempt to lower heating costs, many greenhouse growers consider lowering greenhouse temperatures during the colder months. The feasibility of such a move is totally dependent on the crop in production. Every plant species on earth has an optimum temperature for growth. Additionally, many of the growth stages a plant passes through are affected by temperature. Thus it is extremely important to have a thorough understanding of the temperature requirements for the crop in production.

Each plant species has an optimum temperature for seed germination. This must be considered in setting the greenhouse temperature. Heating cables can be used to warm the germinating media while keeping the greenhouse air temperature cooler, however, the impact of the cooler greenhouse air temperature on seedling growth after germination must also be considered. It is usually not a very good idea to try and conserve heat by lowering the thermostat during germination.

Every plant species has a low temperature at which it stops growing. This temperature is called the base temperature. There is also a temperature, warmer than the base temperature, at which plant growth is at a maximum. This temperature is called the optimum temperature. Plant growth generally steadily increases between the base and optimum temperatures. Above the optimum, growth starts to decrease. In general, plants originating in cooler climates have lower base and optimum temperatures than those originating in warmer climates.

Many species will grow fairly well at temperatures below the optimum. The main consequence of less than optimum temperatures during growth is a delay in development. Thus at lower temperatures each production cycle occupies the greenhouse space for a longer time period. One needs to consider whether or not producing more product in a given time outweighs the savings accrued by lowered heating costs. A major consequence of lowered temperatures is the number of days to flower. Decreasing the temperature by 0.5°C (1°F) delays flowering in petunia by 3 days! If the thermostat was lowered by only 2.7°C (5°F), bloom is delayed by 2 weeks.

Depending on species, lateral branching and the number of flowers often increase with cooler temperatures. In some crops this may be desirable while in others it is not. In some species flowering is inhibited altogether if temperatures are too cool. Thus it is imperative that the temperature requirements for each species in production be thoroughly understood before attempting to save money by reducing the greenhouse temperature.

With cooler temperatures, water evaporates more slowly, thus disease incidence often increases with cooler temperatures, especially with some diseases such as *Botrytis*. Crops also require less water when grown at cooler temperatures. It is a good idea to water cooler greenhouses between 10 a.m. and noon to ensure that excess water evaporates before nightfall. Avoid watering too early in the day, especially if using cold water sources since the water will cool the roots and retard growth. With later watering, the growing media has had a chance to warm up a bit, thus the cooling caused by the cold water is not as drastic.

When the greenhouse temperature is reduced to save fuel, there is a greater difference between the day and night temperatures compared with crops grown at optimum temperatures. This greater difference can lead to greater stem elongation (see the section on DIF which follows this section).

If lowering the greenhouse thermostat is necessary, make sure to consider only crops that grow well under cooler conditions. Trying to grow a crop needing warm temperatures in a cool greenhouse is futile. Some crops to avoid in a cool greenhouse (<20°C(<68°F nights)) include tomatoes (*Solanum lycopersicum*), peppers (*Capsicum annuum*), cucumbers (*Cucumis sativa*), *Alternanthera*, New Guinea impatiens (*Impatiens* × *hawkeri*), *Lantana, Vinca, Celosia, Cleome*, coleus (*Solenostemon* spp.), *Cosmos, Gomphrena, Ipomoea, Melampodium, Portulaca*, and sunflowers (*Helianthus annuum*).

Crops to consider growing in a cool greenhouse include lettuce (*Lactuca sativa*), salad greens, *Argyranthemum, Osteospermum*, annual phlox (*Phlox drummondii*), *Nemesia, Calibrachoa, Diascia*, snapdragon (*Antirrhinum*), *Alyssum, Dianthus*, and pansies (*Viola*). Remember that even though crop quality may not suffer with lower temperatures in these species, production time will be lengthened considerably. It is a good idea to grow even these cool-tolerant crops at optimum temperatures for 2 or 3 weeks after germination to establish a good root system. Only then lower the thermostat.

One way to reduce heating costs is to modify production scheduling, especially in colder climates. By delaying the date for a finished product by only 1 month or so in the spring, huge savings in heating costs can be realized (Runkle, 2012a). For example, changing the date of finishing several floricultural crops from 15 April to 15 May in the northern USA reduced heating costs by 70%! Of course it depends on the species in production and whether or not the finishing date can be moved or not whether this is a viable option. Another course of action to reduce heating costs is to change the crops grown. Selecting a crop with a lower base temperature may allow production of a similarly valued crop at a lower heating cost, compared with a warmer selection. Greenhouse crops can be categorized based on their base temperature as presented in Table 11.2 (after Runkle, 2012a).

While temperature regulates plant growth and development, it should not be considered exclusively when selecting crops or managing them in the greenhouse. Light levels can greatly alter the selection of temperature for production. When light levels are less than optimum, crops should generally be grown at less than optimum temperatures. This accounts for a reduction in photosynthates available for growth due to reduced light levels. If the temperature were maintained at optimum levels, photosynthates would be used for respiration with little left over for growth. Free software is available at www.virtualgrower.net to estimate heating costs for various crops.

Average daily temperature (ADT) is an important concept in greenhouse temperature management. It is an average temperature that considers the length of time each day that a greenhouse is at a specific temperature rather than simply what the high and low set points are. The ADT is important in that plant development generally responds to this value rather than the high and low temperatures of a greenhouse. If plants are not developing fast enough to meet production deadlines, the ADT can be increased. If the crop is developing too rapidly, the ADT can be decreased. The ADT is easily calculated by multiplying the number of hours in each 24 h cycle that a plant is exposed to each set temperature with that temperature, adding them, and then dividing by 24. For example if a crop is exposed to 10 h at 25.6°C, 6 h at 17.8°C, and 8 h at 15.6°C, the ADT would be [(10 × 25.6) + (6 × 17.8) + (8 × 15.6)]/24, or 20.3°C. A grower could compare a new ADT estimate based on proposed thermostat settings for saving fuel and calculate a reasonable estimate of any change in production. An even more precise estimate on the effect changing the temperature would have on production would consist of calculating the heat units needed for production of the crop in question and comparing actual accumulation with the accumulation that would occur with new temperature settings.

Table 11.2. Temperature sensitivity of various greenhouse crops based on their base temperature (after Runkle, 2012a).

Species	Base temperature (°F)
Cold-tolerant plants – base temperature 39°F or lower	
Ageratum, Alyssum, Campanula, Cineraria, Diascia, Easter lily (*Lilium longiflorum*), *Gaillardia, Leucanthemum, Nemesia, Rudbeckia, Scabosia*, Thanksgiving cactus (*Schlumbergera*)	–[a]
Dianthus (Super Parfait series)	39
Marigold (African, Moonstruck series) (*Tagetes erecta*)	37
Marigold (French, Janie series) (*Tagetes patula*)	34
Osteospermum Passion series	35
Petunia (Grandiflora) Dreams series	37
Petunia (Milliflora) Fantasy series	37
Snapdragon Montego series (*Antirrhinum*)	36
Viola Sorbet series	39
Cold-temperate plants – base temperature between 40 and 45°F	
Calibrachoa, Coreopsis	–[a]
Cosmos sulphureus Cosmic series	45
Dahlia Figaro series	42
Gazania Daybreak series	41
Geranium (seed) Florever series (*Pelargonium*)	41
Impatiens (seed) Accent series	43
Lobelia Riviera series	41
Marigold (African) Antigua series (*Tagetes erecta*)	40
Petunia (Spreading) Easy wave series	45
Petunia (Spreading) Wave series	42
Rudbeckia (annual) Becky series	40
Salvia splendens Vista series	45
Verbena Obsession series	44
Verbena Quartz series	41
Wax begonia Sprint series (*Begonia*)	43
Cold-sensitive plants – base temperature of 46°F or higher	
African violet (*Saintpaulia*), banana (*Musa* spp.), *Begonia* (fibrous), *Cladium, Gazania, Hibiscus*, New Guinea impatiens (*Impatiens* × *hawkeri*), pepper (*Capsicum*), *Phalaenopsis* orchid, poinsettia (*Euphorbia pulcherrima*), purple fountain grass (*Pennisetum setaceum*), rose (*Rosa*)	–[a]
Ageratum High Tide series	46
Angelonia Serena series	50
Blue salvia Victoria series	49
Browallia Bell series	48
Celosia Gloria series	50
Pentas Graffiti series	49
Portulaca Margarita series	48
Vinca Viper series	53

[a]Specific base temperatures have not been determined for these crops.

For example, suppose a grower had a floricultural crop with a base temperature of 10°C that required 10 weeks in the greenhouse at an ADT of 20°C. This ADT is achieved with thermostat settings of 12 h at 22.2°C and 12 h at 17.8°C. These values would correspond to heat unit requirements of (20 – 10) = 10 heat units/day × 7 days/week × 10 weeks = 700. Now suppose the grower wanted to investigate changing the temperature regime of the greenhouse to 12 h at 23.3°C (easy to keep the greenhouse this warm during the day) and 12 h at 15.6°C. This would produce an ADT of 19.4°C. To translate this value into a more useful piece of information, the heat units associated with this change would be (19.4 – 10) = 9.4 heat units/day. We need 700 to bring the crop to harvest, thus we

need 700/9.4 which is ~74 days at this temperature regime. It would take 4 more days to produce the crop, but we would save quite a bit in lowering the night temperature to 15.6°C.

Day/night temperature difference (DIF)

Recall the concept of DIF that was presented in Chapter 9 of this volume. DIF is the difference between the day temperature and the night temperature, and it is an extremely important management tool for greenhouse growers. Managing DIF reduces a grower's dependence on applied growth regulators for managing stem elongation in many greenhouse crops.

If days are warmer than the nights, a positive DIF (+DIF) exists, and if the night is warmer than the day, a negative DIF (-DIF) exists. Stem elongation is enhanced with a more positive DIF, and plants remain short statured if the DIF is around zero or negative. By controlling DIF, growers can manipulate the size of their plants, but only to the extent that a species responds. Some species that exhibit a large response to DIF include Easter, Oriental and Asian lilies (*Lilium* spp.), *Dianthus, Chrysanthemum*, tomato (*S. lycopersicum*), poinsettia (*Euphorbia*), green bean (*Phaseolus vulgaris*), *Salvia*, watermelon (*Citrullus lanatus*), *Celosia*, sweet corn (*Zea mays*), *Fuchsia, Impatiens, Portulaca, Gerbera, Petunia*, snapdragon (*Antirrhinum*), geranium (*Pelargonium*), and rose (*Rosa*). Species with little to no DIF response include squash (*Cucurbita* spp.), *Platycodon*, French marigold (*Tagetes patula*), tulip (*Tulipa*), hyacinth (*Hyacinthus orientalis*), *Narcissus*, and *Aster*. The DIF response is observed when the plant is normally undergoing significant stem elongation. If a grower knows the crop growth characteristics well, they can time the DIF to occur only during the period of most significant stem elongation and not the entire growth cycle.

In general a DIF of -5°C is sufficient to induce shorter internode length and therefore shorter plants. If the DIF is too negative, undesirable responses such as chlorosis may occur. In addition, growers need to be cautious when using DIF as a method of growth regulation since the rate of crop development is affected by temperature as well. Any DIF treatment that results in an increase in the ADT will be likely to result in accelerated crop development and any treatment that reduced the ADT would retard growth and development.

In order to achieve a negative DIF, significant greenhouse heating at night is needed. However, lowering the greenhouse temperature below the night temperature for 2 h at sunrise (which creates a negative DIF) is just as effective as maintaining the negative DIF with heating for the entire night. This procedure is called the 'cool morning pulse'. It reduces the need for excessive heating during the night to maintain a negative DIF.

The DIF response may be a response to gibberellin production. Warmer days and cooler nights (+DIF) often stimulates internode elongation by enhancing gibberellin synthesis or action.

Floral initiation and development

Flower bud initiation and development of many greenhouse grown crops is highly regulated by temperature. Two types of temperature regulation of flowering are observed in sensitive species. A qualitative response is observed in species that have an absolute requirement for a specific number of days at a precise temperature for flowering. Plants with a quantitative requirement are those where the flowering response may be modified by changing the temperature, but temperature itself does not determine whether or not the plant will flower. These types of plants will eventually flower regardless of temperature, but temperature regulates at what stage of development flowering will occur.

An example of the quantitative flowering response can be observed in day-neutral strawberries (*Fragaria* × *ananassa*); they eventually flower no matter what temperature they are gown at (within reason of course), however, they flower more rapidly when grown at cooler temperatures. An example of a qualitative flowering response is vernalization (studied in Chapter 9 of this volume) and observed in many flowering bulbs. Some species that are not bulbs have a qualitative low temperature requirement for flowering. These species include *Cineraria, Calceolaria, Hydrangea*, and *Cymbidium* orchids. Other species have a qualitative high temperature requirement for flowering and include Azalea (*Rhododendron* spp.), *Clarkia*, and annual Larkspur (*Consolida*).

Temperature and greenhouse vegetable production

In many greenhouse vegetable-production operations, temperature can greatly influence productivity not only by its effects on general growth rates, but by specific effects on certain stages of development. In tomato (*S. lycopersicum*), for

example, fruit set is greatly reduced when day temperatures are above 32.2°C, and nights are above 23.9°C or below 13.9°C. Prolonged growth of cucumber (*C. sativus*) at temperatures above 29.4°C leads to reduced fruit quality. Lettuce (*L. sativa*) grown at excessively warm temperatures tends to become bitter and flower prematurely. Additionally, lettuce seed will not germinate if temperatures are too high.

Light

The lighting environment of a greenhouse is crucial to its success or failure. Both the quantity and the quality of light utilized in a greenhouse can impact plant growth and development. When evaluating the light levels in a greenhouse, two sources of light must be considered: (i) natural; and (ii) artificial. Photoperiod is another important aspect of greenhouse lighting. Many species respond to daylength during their growth and development and certain developmental stages such as flowering may be highly regulated by photoperiod. It is important to distinguish between effects due the length of the daily light period and effects due to the length of the dark period. Responses due to the length of the dark period are true photoperiodic effects and are controlled by the light-sensing pigments especially phytochrome.

Both quantity and quality must be taken into consideration when discussing light with respect to plants and their responses. Even though light levels are often reported as foot candles, lux, etc., the most appropriate measurement for light levels associated with growing plants is micromoles of photosynthetically active radiation (PAR) per square meter per second (μmol PAR/m^2/s). In greenhouse production, it is often best to consider the daily light integral (DLI) which is the daily total of photosynthetically active light (PAR, 400–700 nm) measured in moles per square meter per day (mol/m^2/day). During the summer on a cloudless, long day, mid-latitudes receive approximately 60 mol/m^2/day. In contrast, during a short, cloudy, winter day, less than 5 mol/m^2/day might be received on a greenhouse bench. This is important in that the DLI is directly related to plant productivity and therefore yield. If an insufficient DLI is received by a crop, productivity will suffer, perhaps even to the point of crop failure.

A general goal DLI for many greenhouse situations is 10–12 mol/m^2/day. Sensitive or shade-tolerant crops such as African violets (*Saintpaulia ionantha*) or *Impatiens* grow well at half that amount. While there are maps that provide estimates of DLI for various times of the year at different locations, it is best to measure the actual DLI yourself. The investment in a good light sensor is well worth it.

Natural

Natural light is provided by the sun. Only a portion of solar radiation striking the greenhouse actually reaches the plants inside the greenhouse. The quantity and quality of that light are affected by: (i) time of year; (ii) time of day; (iii) prevailing weather conditions; and (iv) greenhouse covering material. In general a grower is seeking to maximize the amount of natural light reaching the plants inside his or her greenhouse. During winter when the greenhouse is most likely being used to force an out-of-season crop, lighting may pose a double problem. Depending on longitude, days may be very short and light levels very low due to the low declination of the sun during winter in many climates. Even though natural light levels may be at an unacceptably low level during winter, there are a number of things a greenhouse manager can do to enhance the lighting inside the greenhouse.

Aim for about 50% light transmission into the greenhouse during the winter months (Runkle, 2012b). Invest in a reliable light meter, preferably one that measures PAR so that you really know how much light there is in the greenhouse. Once you have a good light meter, understand how to use and maintain it. When taking measurements, make sure the sensor is perfectly level, at canopy height and not in a shadow. If the sensor is permanently mounted, make sure it is not in the path of traveling shadows during the day and try to keep it dry. Even though the sensor may be waterproof, water droplets may contain dissolved solids that could build up over time as the droplets dry on the sensor which could lead to faulty readings. Consult the manufacturer for how often the sensor should be recalibrated.

To maximize light entering the greenhouse, make sure the greenhouse covering is clean, and if it can't be cleaned, make sure it's renewed for the following winter season. In addition, try using anti-condensation materials for the inside of poly covers to minimize light absorption or reflection by water droplets. Make sure there aren't too many hanging baskets overhead as they can significantly reduce the light hitting bench crops.

Above all, understand the lighting requirements of the crop you are growing. Know what the DLI requirement is to bring your crop to a salable condition and investigate whether or not photoperiodic stimulation is warranted. Don't try to grow a crop that you are not equipped to produce. If natural light levels are too low and you can't afford supplemental lighting, consider changing which crop you'll grow.

Supplemental lighting

Since many greenhouse crops are produced when the natural DLI is below an optimum level, supplemental lighting is required to enable production. When thinking about supplemental lighting for your greenhouse, remember there are two main reasons to consider it. One is to supplement the natural DLI to enhance photosynthesis and thereby enhance plant growth and productivity and the other is for photoperiodic stimulation. Be sure you understand which of the two you are dealing with. In many cases, you will probably be concerned with both.

Photosynthetic needs

CROP LIGHT REQUIREMENTS Every greenhouse crop whether flower, fruit, herb or vegetable has a specific requirement for a minimum DLI needed to bring the crop to market. By knowing the light requirements for your crop, you will know how feasible it is to provide supplemental lighting economically. For most crops, increasing the DLI decreases the time to flower and increases plant quality (numbers of flowers or fruit, improved branching, thicker stems), but only up to a point. The point at which increasing the DLI has no positive effect on a crop varies among species and is called the saturation DLI. By understanding the crop you are growing, you would know if increasing the DLI is worth it. In general, increasing the DLI above 15 isn't warranted unless you are dealing with a specialty crop where you know there is a benefit.

Greenhouse lettuce (*L. sativa*) production requires a minimum of 12–13 mol/m^2/day, sweet pepper (*C. annuum*) needs at least 12 mol/m^2/day while tomatoes (*S. lycopersicum*) require at least 6 mol/m^2/day for seedling production ultimately increasing to at least 30 mol/m^2/day for fruit production (Dorais, 2003). Cucumber (*C. sativus*) production requires at least 5.5 mol/m^2/day but fruit development can be reduced from 24 days at 5.5 mol/m^2/day to 10 days at 30 mol/m^2/day (Dorais, 2003).

A second consideration when increasing the DLI is to take into account any effect the lighting for enhanced DLI might have on a photoperiodic response. For example, supplementing light to enhance flowering of short-day plants might be offset by the delay in flowering caused by longer days under supplemental lighting to enhance DLI. Some estimates of saturation DLI values are given in Table 11.3.

LIGHT SOURCES, TIMING, DURATION, AND INTENSITY The high pressure sodium lamp is still the recommended light source for increasing the DLI (Runkle, 2012b). As technology of light emitting diodes (LED) improves, this recommendation may change. However, LED units are much too expensive still to warrant their use in commercial settings.

To determine the amount of supplemental lighting required for any specific situation, verify the DLI needed to bring the crop to market. The Internet provides a rich source of references for determining this value. Once this value is derived and the lamp source, number of units, and light supplied per unit are known, the duration needed to supply the needed radiation can be determined.

There are many options available when selecting supplemental lighting configurations for greenhouse production. As a point of reference, let's assume we are using high pressure sodium lamps that can provide 150 μmol/m^2/s PAR. Our estimated DLI is 4 mol/m^2/day. We want to grow lettuce (*L. sativa*) which requires a minimum of 12–14 mol/m^2/day, thus we need to supply at least 12–4 = 8 mol/m^2/day. Lights providing 150 μmol/m^2/s PAR would provide 540,000 μmol/m^2/h PAR (150 × 60 × 60) which is 0.54 mol/m^2/h PAR. To determine the length of time the lights must be illuminated, divide the supplemental lighting molar requirement by the per hour molar light production. For example, if we need 8 mol/day extra illumination and our lighting system supplies 0.54 mol/m^2/h PAR, we would need 14.8 h (8/0.54) of illumination per day to meet the minimum requirements. If we needed 14 mol/m^2/day, we would need to provide 10 mol/m^2/day extra or 18.5 h (10/0.54) of supplemental illumination.

Longer light periods at lower illumination tend to increase productivity compared with shorter

Table 11.3. Saturation daily light integrals (DLI) for a number of bedding plants normally produced in the greenhouse (adapted from Runkle, 2012b).

Crop	Estimated saturation DLI (mol/m^2/day) PAR
Angelonia Serena series	5
Browallia Bell series	11
Celosia Gloria series	10
Cosmos sulphureus Cosmic series	4
Dahlia Figaro series	11
Dianthus Super Parfait series	6
Gazania Daybreak series	20
Geranium (seed) (*Pelargonium*) Florever series	12
Impatiens (bedding) Accent series	<4
Lobelia Riviera series	5
Marigold (African) (*Tagetes*) Antigua series	5
Marigold (African) (*Tagetes*) Moonstruck	8
Marigold (French) (*Tagetes*) Bonanza series	6
Marigold (French) (*Tagetes*) Janie series	6
Osteospermum Passion series	12
Pentas Graffiti series	7
Petunia (Grandiflora) Dreams series	7
Petunia (Milliflora) Fantasy series	17
Petunia (Spreading) Easy wave series	9
Petunia (Spreading) Wave series	9
Portulaca Margarita series	8
Rudbeckia (annual) Becky series	7
Salvia (blue) Blue Bedder	10
Salvia (red) Vista series	10
Snapdragon (*Antirrhinum)* Montego series	12
Verbena Obsession series	10
Verbena Quartz series	13
Vinca Viper series	5
Viola Sorbet series	>12
Wax begonia (*Begonia*) Sprint series	7
Zinnia Dreamland series	8

durations of higher intensity light. Continuous illumination often leads to metabolic perturbations such as chlorosis and reduced growth (Sysoeva *et al*., 2010). In general, if photoperiod is not constrained by other physiological responses to it, supplemental lighting is often provided for 10–20 h to provide the required DLI.

Computer programs have been created to monitor the DLI and adjust supplemental lighting accordingly. Lights may be turned off if the DLI is reached. The decision to limit the DLI is important in those crops for which a saturation DLI has been established.

Photoperiodic needs

As you move farther away from the equator, the length of the daily light period or photoperiod dramatically changes over the course of a year. Daylength at the equator is always 12 h. In either hemisphere, as the season changes from summer into winter, days become increasingly shorter until the winter solstice on 21 or 22 December (depends on the year), when at any given point between the equator and the corresponding North or South Pole, the daylength is at a minimum. After this date, daylength gradually increases until the summer solstice on 20 or 21 June when the daylength is longest. The process then begins all over again.

Plants readily detect changes in the length of the day via the pigment phytochrome. Sunlight favors the formation of the far-red form of phytochrome (P_{fr}). During the dark, P_{fr} gradually reverts to the red form of phytochrome (P_r). True photoperiodic plant responses are regulated by the relative amounts of

P_{fr} and P_r remaining after the dark cycle. Shorter dark cycles result in a greater amount of P_{fr} remaining with less P_r. Thus a long-day response is actually a response to a short dark cycle and an elevated level of P_{fr}.

Plant leaves absorb more red light than far-red light. Sunlight has a red:far-red light ratio of 1.2, thus there is a little more red light than far-red light in sunlight, so the balance of P_r:P_{fr} favors P_{fr}. At the end of the day, much of the phytochrome in a plant is in the physiologically active P_{fr} form. Phytochrome in the physiologically active form (P_{fr}) promotes shorter, highly branched plants with small thick leaves. Leaves intercepting light that has passed through a leaf is receiving light with a red:far-red ratio of 0.13! This light has a large amount of far-red light in it, thus the favored form of phytochrome in this instance would be P_r, the physiologically inactive form. This leads to a preponderance of phytochrome in the red form, which is the physiologically inactive form. With P_{fr} is lacking, as is the case in an overcrowded greenhouse or on a greenhouse bench shaded by hanging baskets, plant stems elongate, leaves grow larger and thinner, and branching is greatly reduced. The 'shade avoidance response' can be attributed to a lack of phytochrome in the physiologically active form, P_{fr}.

One of the most important plant responses to photoperiod is flowering and many greenhouse crops are photoperiodically sensitive with respect to flowering (Table 11.4). With respect to flowering plants are day-neutral, short-day or long-day plants.

Day-neutral plants are not sensitive to daylength when it comes to flowering. Some common greenhouse crops that are day-neutral include African violets (*S. ionantha*), cucumbers (*C. sativus*), rose (*Rosa*), and tomatoes (*S. lycopersicum*).

Short-day plants are those species that will only flower or flower more rapidly after exposure to days that are shorter than a defined critical photoperiod. The critical photoperiod is often different for different species, but they all must be exposed to days shorter than their critical photoperiod before they will flower. Some short-day crops include poinsettia (*Euphorbia*), and *Chrysanthemum*.

Conversely, long-day plants will only flower or flower more rapidly after exposure to days that are longer than a critical photoperiod. As in short-day plants, the critical photoperiod is often different for different long-day species, but they all will only flower after exposure to days longer than the critical photoperiod. Some long-day species include *Rudbeckia*, lettuce (*L. sativa*), and spinach (*Spinacia oleracea*).

Some species such as strawberry (*Fragaria* × *ananassa*) include cultivars that are day-neutral, short-day or long-day.

In addition to the daylength, the number of cycles required by individual species varies from as little as one to as many as 14 or more. The intensity of flowering is often related to the number of photoperiodically inductive cycles a plant has been exposed to. Profuse flowering often follows exposure to several or to many cycles while sparse flowering follows exposure to only a few. If plants are exposed to too many inductive cycles, they may become dormant. To complicate matters even more, temperature can moderate the response to

Table 11.4. Photoperiodic requirements for flowering of some major greenhouse crops (adapted from Runkle, 2012b).

Crop	Day-neutral	Short-day	Long-day
Begonia	X		
Dahlia		X	
Geranium (*Pelargonium*)	X		
Impatiens	X		
French marigold (*Tagetes*)	X		
Pansy (*Viola*)			X
Petunia			X
Snapdragon (*Antirrhinum*)			X
Black-eyed Susan (*Rudbekia*)			X
Campanula			X
Columbine (*Aquilegia*)	X		
Coreopsis			X
Hosta			X
Lobelia			X
Shasta daisy (*Leucanthemum*)			X
African violet (*Saintpaulia*)	X		
Chrysanthemum		X	
Cyclamen	X		
Poinsettia (*Euphorbia*)		X	
Rose (*Rosa*)	X		
Strawberry (*Fragaria*)	X	X	X
Tomato (*Solanum*)	X		
Cucumber (*Cucumis*)	X		
Pepper (*Capsicum*)	X		

photoperiod. In general, cooler temperatures mimic shorter days while warmer temperatures mimic longer days.

Besides flowering, photoperiod also influences vegetative growth, plant height, branching, and other general growth characteristics.

Greenhouses are often used to produce commodities out of season. Often the natural daylength is not suitable for certain aspects of a crop's production cycle and may need to be altered. Daylength may need to be either shortened or lengthened depending on the crop and time of year.

DECREASING THE PHOTOPERIOD When photoperiods are too long, they can be artificially shortened by draping black plastic or black cloth over plants at a pre-specified time during the day followed by appropriate removal (Fig. 11.4). Shorter days may be desired to induce flowering in short-day plants or prevent flowering in long-day plants. The black cloth or plastic should not be placed over the plants too early in the day or removed too late the following day or excessive heat may build up. The covering must be complete since even small amounts of light entering through slits or cracks can be perceived by plants. Even under natural short days, growers should be mindful of light from parking lots or other sources of light that might be perceived by plants in the greenhouse. When particularly sensitive species are grown, black cloth or plastic may be used even under natural short days to ensure that plants are in absolute darkness during the nyctoperiod.

LENGTHENING THE PHOTOPERIOD Under naturally short days, the daylength can be lengthened for a photoperiodic response using the night interruption (NI) technique (Fig. 11.5). This red-light effect on phytochrome was described in detail in Chapter 8 of this volume. In general, this technique uses low level light applied for 3–4 h during the middle of the normal dark period. Alternatively, the daylength can be extended with similar lighting from sunset until the desired daylength is reached. In either approach, the light source should provide at least 10 foot candles. Incandescent lamps are often used to elicit the daylength response since they are inexpensive and easy to use. They do have the drawback of emitting significant far-red light which often promotes stem elongation in many species. The red:far-red ratio of light emitted by incandescent lights is 1.07 (Downs and Thomas, 1982) thus even though they do emit far-red light, enough red light is emitted to favor a preponderance of P_{fr} at the end of the interruption. Recall that the idea behind the NI or daylength extension with low level lighting is to convert P_r to P_{fr} such that an effectively high enough level of P_{fr} remains at the end of the dark cycle. In the case of the NI technique, we want a high level of P_{fr} at the end of the dark cycle that follows the interruption.

To avoid stem elongation, widely spaced sodium or metal halide lamps set high above the plant canopy can be used to provide the low level of light desired without inducing stem elongation.

Water

Water management is crucial for successful greenhouse management. Besides the obvious need to supply plants in a greenhouse with water since they are not exposed to natural rainfall, management of the water vapor in the air of a greenhouse is one of the most important and difficult attributes of greenhouse management. Temperature and light levels can be automatically adjusted with the help of automation. Humidity regulation is more complex. In order to understand greenhouse humidity management techniques, a thorough understanding of air water vapor is important.

The water vapor content of air is often expressed as RH (relative humidity). RH is the ratio of the weight of water in a unit volume of air to the water holding capacity of that unit volume of air at a specific temperature and pressure. The pressure of the air in a greenhouse does not change that rapidly or dramatically, therefore its influence on RH is minimal. Temperature is another matter. It can change rapidly and dramatically and has a large impact on the RH of the air in a greenhouse. It is this influence of temperature on RH that makes it particularly difficult to control.

As air warms, it can hold more water. Air at 21°C can hold twice the water of air at 10°C. Warming a specific volume of air without changing the absolute amount of water vapor in it reduces its RH. Cooling the same parcel leads to an increase in RH. Even though the RH changes with temperature, the amount of water in the given volume of air is not changing.

The dew point (wet bulb temperature) is another important characteristic of a parcel of air. It is the temperature at which water will condense out of the air. As soon as an object reaches

(a)

(b)

Fig. 11.4. Strawberry (*Fragaria* × *ananassa*) plug plants before being placed in the greenhouse for winter (New Jersey, USA) production. (a) Prior to short-day photoperiod treatment. (b) Receiving short-day photoperiod treatment by being draped in black plastic.

the dew point, water will begin to condense on it. The dry bulb temperature of the air is the commonly measured air temperature. Dew point is directly related to RH. When the RH is high the dew point is very close to the dry bulb temperature. At a lower RH, the dew point is much lower than the dry bulb temperature. For example, if the RH is 85% and the dry bulb temperature is 15.5°C, water will condense on objects at 12.7°C. Increase the RH to 95% (which is easy to do in a greenhouse at night) and water condenses on objects at 15°C.

Fig. 11.5. Strawberries (*Fragaria* × *ananassa*) grown during December (New Jersey, USA) using the night interruption (NI) technique with incandescent lighting to promote inflorescence elongation and flowering, both long-day responses. Note the incandescent bulb at the top middle of the photo.

While humidity deficits may occur in greenhouses in some arid regions, the RH inside a well-constructed greenhouse can usually be increased with misting or humidifiers. Briefly wetting the floor also helps raise humidity, however, free-standing water is not desirable. More often than not, problems in a greenhouse occur as excessively high RH at night. High RH at night in a greenhouse is a problem due to condensation.

Even a small difference in RH, in the previously discussed case 85% versus 95%, can make a big difference in the amount of water that can condense on plant leaves and the temperature at which it condenses. Again, this is especially important at night. During the day the dry bulb temperature is almost always higher than the dew point, except on cloudy, rainy days. Thus condensation is usually not much of a problem during the day. But why is condensation at night a problem? The answer is disease control. Condensed water on leaves is a perfect medium for the germination of fungal spores that cause significant disease problems in greenhouse crops. In addition, dripping or splashing water spreads spores throughout the greenhouse.

Botrytis and powdery mildew (*Erysiphe, Leveillula, Microsphaera*, and *Spaerotheca*) are two important greenhouse disease problems which may be hard to control under humid conditions. To control disease development in the greenhouse, the RH must be controlled. There are a number of cultural practices which can greatly reduce problems associated with high night-time RH. Remember, even a small difference in the RH can make a big difference in the temperature at which water condenses out. A key to good greenhouse RH management is to enter the night with the greenhouse as dry as possible.

Proper greenhouse watering is important. Good floor drainage prevents water from puddling and watering early in the day allows excess water to evaporate and be removed from the greenhouse atmosphere via ventilation during the day. Proper plant spacing on mesh benches allows good air movement within the greenhouse which helps to keep the RH a bit lower. Canopies that are too close trap transpired water and raise the RH immediately around the plant. When watering, apply only enough water to thoroughly moisten the growing medium. Avoid wetting the foliage if possible. Watering too heavily can create puddles of standing water and also it leaches nutrients from the soil mix. Excessive watering can also lead to waterlogged root systems, resulting in poor growth, root rot, and epinasty. Keep weeds out of the greenhouse. Besides being unsightly and potential sources of disease and insect contaminants, weeds transpire water from the soil into the air.

Heating below the plants via cables or hot water/steam pipes increases air circulation around the plants as the air rises from the heat source, plus it increases the surface temperature of plant tissues, potentially preventing condensation on them.

If the greenhouse is a poly cover, using a cover with a wetting agent incorporated into it or spraying a wetting agent on an existing cover helps moisture that condenses drain down the curve of the covering rather than drip onto the plants below. If the cover is rigid such as glass, make sure the roof is pitched steeply enough to ensure good drainage rather than dripping.

Air movement within the greenhouse with overhead poly tubes or horizontal air flow fans (see earlier in this chapter) helps mix the air and maintain a lower RH. Mixing air removes cold pockets where moisture could more easily condense and also facilitates mixing of the air around plant canopies.

Heating and ventilation are two powerful tools in controlling greenhouse humidity. Heating raises the air temperature which raises the amount of water the air can hold before condensing out while ventilation exchanges drier air from outside with moist air from inside the greenhouse. Neither alone

is sufficient in controlling humidity, but used together, they can help regulate humidity at acceptable levels.

The approach to humidity control with heating and venting depends on the greenhouse set up. In houses with passive venting (no fans) such as smaller, homeowner units, the heat should be turned on for a while, then the vents opened to allow heated, rising air to exit the greenhouse. Cooler drier air from outside will replace this air, reducing the RH inside.

With forced ventilation greenhouses (houses with fans), operate the ventilation fans for a few minutes to remove humid inside air with drier outside air. Once the fans are turned off, the heat is turned on to warm up the outside air that has been drawn into the greenhouse. The key is to operate the vents and the heat separately; they should not be operating at the same time. This venting-followed-by-heating process should be performed several times an hour for several hours after sunset and several hours before sunrise. The length of time each venting cycle is on depends on the exchange rate of the venting system. One full exchange of greenhouse air is desired per cycle. High capacity systems may only require venting for 2 or 3 min each cycle while a lower rate system may take much longer.

A good hygrometer or a sling psychrometer is a wise investment for measuring humidity levels in the greenhouse. A general guideline for desirable humidity levels is based on the observation that plants can tolerate a higher RH at warmer temperatures. The RH in the greenhouse should be no higher than 83% if the greenhouse temperature is 10°C, 89% at 16.1°C, 91% at 20°C, and 95% at 30°C (Prenger and Ling, 2000). Besides disease problems associated with high RH, reduced transpiration by plants under high humidity may lead to reduced nutrient uptake and therefore smaller, weaker plants.

Carbon Dioxide Enrichment

Carbon dioxide (CO_2) is a major substrate for one of the most important biochemical processes on our planet: photosynthesis. The average CO_2 concentration in the atmosphere has risen from 382 ppm in 2007 to 392 ppm in 2012, or about 2 ppm/year (Tans, 2012). While elevated atmospheric CO_2 levels are a concern of global climate change, elevated levels of CO_2 are often desirable in greenhouse environments to increase productivity. Enhanced CO_2 levels in the greenhouse nearly always results in enhanced yield and or quality of crops grown in them. This is especially true in winter greenhouse production situations in regions with short days and naturally low light intensities. Combining CO_2 enrichment with supplemental lighting has a synergistic effect (Dorais, 2003).

The reason CO_2 enrichment works centers around a key enzyme involved in photosynthesis, RuBisCo. Recall that RuBisCo is a dual function enzyme, it can fix CO_2 (desired) or O_2 (not desired). The main effect of CO_2 enrichment is to shift the balance between carboxylation and oxygenation via RuBisCo towards carboxylation (Tremblay and Gosselin, 1998). This effect is consistent over a wide range of light levels, indicating that CO_2 enrichment is effective at any time of the year. The optimum level of CO_2 in greenhouses lies between 700 and 900 ppm. In general, the greatest benefits to CO_2 enrichment are observed in the vegetative growth of young seedlings (Kimball, 1983).

C3 plants generally show a greater growth response to elevated CO_2 levels compared with C4 plants (Prior *et al*., 2011). This is not surprising since C3 plants experience photorespiration and C4 plants do not, and carboxylation rather than oxygenation by RuBisCo is enhanced by elevated CO_2. Besides reduced photorespiration under elevated CO_2 levels, enhanced net photosynthesis also results in both C3 and C4 species. There is generally a 33–40% increase in net photosynthesis for C3 species and a 10–15% increase for C4 species (Kimball, 1983).

In addition to stimulated photosynthesis, elevated CO_2 leads to altered carbon partitioning towards below-ground organs which leads to an increased root:shoot ratio. Plants will often allocate carbon towards tissues responsible for acquiring the limiting component(s) of a metabolic process. Under conditions of elevated CO_2, water and nutrients are limiting photosynthesis, thus carbon is allocated to roots to enhance water and nutrient uptake. The extra carbon allocated to roots can be stored for utilization by the plant when carbon may become limiting (i.e. under stress conditions), or the extra carbon may be allocated for increased root growth. Root colonization by mycorrhizae which enhance water and nutrient uptake increases with elevated CO_2.

Most greenhouse crops are grown in containers that limit root growth, thus the response to elevated

CO_2 might be limited compared with plants with unrestricted root systems. Even with the limitations imposed by containers of greenhouse-grown plants, CO_2 enrichment is advantageous in that it allows plants to reach a marketable size more rapidly compared with plants grown under ambient CO_2 levels.

CO_2 enrichment not only enhances photosynthesis, it decreases transpiration rate thereby increasing plant water use efficiency (WUE) (Woodrow *et al*., 1987) and enhancing subsequent drought tolerance. Enhanced seedling growth in the greenhouse induced by CO_2 enrichment translates into healthier, more productive plants in the production field that tolerate transplanting shock rather well (Tremblay and Gosselin, 1998). Enhanced seedling growth in the greenhouse renders plants ready for transplanting in less time, thus freeing up valuable greenhouse space.

Elevated CO_2 leads to more efficient water relations in both C3 and C4 plants by reducing transpiration induced by partial stomatal closure. Reduced transpiration combined with increased photosynthesis leads to enhanced WUE (the ratio of carbon fixed per unit of water transpired). On average WUE is doubled when CO_2 is doubled. Elevated CO_2 may reduce drought stress, however, much of the work to suggest this is in greenhouses or growth chambers with artificially induced drought conditions.

The increased WUE may be offset by increased water use of larger plants caused by elevated CO_2 levels. With enhanced WUE, plants may need watering less frequently under elevated CO_2 environments. As water rights become increasingly important, reduced frequency of watering would be a major benefit for greenhouse managers.

The most common method of enriching the greenhouse atmosphere with CO_2 is by controlled burning of a hydrocarbon, often propane, butane, alcohol, or natural gas. One of the problems with burning a hydrocarbon fuel is the production of carbon monoxide (CO) and ethylene under conditions of incomplete combustion. Incomplete combustion is most often associated with poor burner calibration or low oxygen levels. CO is toxic at very low levels to both plants and humans and ethylene is a plant growth regulator. If a hydrocarbon is burning with a blue or whitish flame it is burning with nearly complete combustion with little or no CO/ethylene generation. If the flames are yellow, orange or red, incomplete combustion is occurring and CO and/or ethylene are being generated. Other byproducts of CO_2 generation by hydrocarbon burning are heat production of water vapor. The quantities of heat, water vapor and CO_2 produced per unit of fuel vary with the fuel being burned and the rate of burning.

The second most common method of CO_2 enrichment is via injection of pure CO_2. Compressed CO_2 comes in metal cylinders or tanks holding 20 or 50 lbs of CO_2 under high pressure (1600–2200 psi). To utilize this method of CO_2 enrichment, an appropriate set up including the tank of gas, pressure regulator, flow meter, valves, and controller is needed. The system must be calibrated to deliver the appropriate amount of gas for the greenhouse volume being enriched during the photosynthetic period. Once the initial control equipment is purchased, this type of system is fairly economical to operate.

Composting organic matter and animal manures has been suggested as a way of economical and sustainable CO_2 generation, however, widespread commercial adoption of these approaches have not yet occurred.

12 Seeding and Seedling Establishment

Many crops begin the production cycle as a seed. Some crops are directly planted in the production field while others are started in a more protected environment such as a greenhouse, high tunnel or cold frame. In all cases, a productive crop must begin from viable, pure, high quality seed. While no standardized international laws to ensure that only high quality, true-to-name seed is sold to growers, many countries have laws regulating seed commerce. Within these laws, issues of seed purity, viability and other measures of quality are addressed. Since it would be impossible to examine all the seed laws in various countries, this section will be limited to a discussion of seed quality issues.

Seed Viability

The most widely accepted measure of seed quality is viability which is measured by testing germination. General rules for seed testing have been adopted by many organizations and usually include at least the following:

1. Four 100 seed samples should be tested for each sample.
2. A suitable substrate such as blotters, towels, sand, etc. should be used as a germination medium.
3. Depending on the test species, seed should be germinated at 20/30°C night/day temperature or 20°C or 15°C constant temperature for a specific duration.
4. Light conditions for germination are also species dependent, as is KNO_3 treatment.
5. Some seeds may need pre-chilling for a specific number of days at 5 or 10°C.
6. Some seeds (onions (*Allium cepa*), asparagus (*Asparagus officinalis*), carrots (*Daucus carota*), buttercups (*Ranunculus*), poppies (*Papaver*), columbine (*Aquilegia*)) may need to mature before testing to allow embryos to complete development.
7. Normal seedling status is based on an accepted definition for the species as well as published drawings for comparison.

A germination test does not totally express viability. It merely estimates the percentage of seeds capable of growing under the prescribed conditions. Some seeds of the lot may be alive but still dormant. To clarify the viability/germination/dormancy status of a seed lot, particularly species that exhibit strong seed endodormancy or to estimate viability alone, seed technologists use the tetrazolium (TZ) test (Peters, 2000).

Imbibed seeds are soaked in a solution of 2,3,5-triphenyl tetrazolium chloride (TTC) dissolved in water. In some species, seeds are cut or seed coats removed to facilitate testing. Living tissues of the seed produce hydrogen (H^+) ions that convert the TTC molecules to an insoluble red dye called formazan. Dyed tissue indicates living, viable tissue. Results are ready in a few hours or a day or two, depending on species.

The percentage viability is indicated by the percentage of seeds exhibiting a red color. When coupled with a standardized germination test, an estimate of the percentage of dormant seeds in the lot can be estimated by subtracting the percentage germination from the percentage viable seed obtained with the TZ test.

The main drawback of the TZ test is that it is more labor intensive than a simple germination test. Additionally, most seed laws do not allow a TZ test as a substitute for a standard germination test.

Seed Purity

Purity is an important attribute of any seed sample. The purity of a seed lot describes its physical composition, verifying that it is the species in question and describing any other crop seed, weed seed, and noxious weed seeds that might be in the sample. It also identifies soil, insects, plant material, and any

other foreign material that might have contaminated the sample. Purity analysis also provides a measure of confidence to the grower that what they have purchased is true to name and unadulterated.

Identification of the amount of pure live seed (PLS) in the sample allows a grower to adjust seeding rates for maximum productivity and utilization of land.

The purity test examines 2500 seeds to establish purity and 25,000 seeds to estimate noxious weed contamination. The sample is examined by hand and sorted into its component parts: (i) pure seed; (ii) other crop seed; (iii) weed seed including seeds, florets, bulblets, tubers or sporocarps of plants normally considered weeds; and (iv) inert matter. Noxious weeds vary from region to region and are categorized as restricted or prohibited. The presence of prohibited noxious weeds renders the entire lot unfit for sale. Restricted weeds found must be listed on the seed label.

Seed Purity and GMOs

As more and more growers utilize genetically modified organisms (GMO) as their choice of seed, conventional and organic growers must be given a mechanism for ensuring that their crops are not contaminated with GMOs. The 'unavoidable' contamination of normal seed lots with GMO seed is called adventitious or technically unavoidable mixing and leads to the production of an admixture. The ability of farmers to choose conventional, organic or GMO seeds with a specified level of purity is called coexistence, and is becoming increasingly difficult (Endres, 2005).

Admixtures can develop due to pollen drift, commingling during harvest or transportation and storage, or from volunteer plants from previous growing seasons. Although precautions are taken to avoid the production of admixtures, a number of historical contaminations have occurred and for many growers, are as unacceptable as contamination with a chemical toxin.

One notable admixture controversy was the StarLink corn (*Zea mays*) fiasco of the late 1990s. StarLink corn were varieties of Bt corn patented by Aventis Crop Sciences, now a part of Bayer AG that was intended for animal feed only. Bt is a naturally occurring soil bacterium, *Bacillus thuringiensis*, and the gene inserted into StarLink corn from the Bt produces a protein, the Bt delta endotoxin, that kills Lepidoptera larvae, most notably, the European corn borer (*Ostrinia nubilalis*). Bt corn is used as an alternative for insecticidal control of the European and south-western corn borer (*Diatraea grandiosella*). The restriction on StarLink corn was due to a possible allergic reaction by some individuals to the specific strain of the delta endotoxin produced in the StarLink corn. StarLink corn was found in food destined for humans and around 30 people had allergic reactions related to eating contaminated corn products. However, the US Centers for Disease Control found no evidence that allergic reactions were due to the StarLink corn. Even so, consumers have a right to know where their food is coming from and what is in it. Since 2004, no corn samples have been found to be contaminated with StarLink corn.

Another admixture incident occurred when volunteer genetically altered corn plants were harvested with non-GMO soybeans contaminating 500,000 bushels of soybeans. The USDA and the Food and Drug Administration (FDA) ultimately received $250,000 in penalties form Prodigene Inc., the company responsible for the mishap, to defray the cost of removing the contaminated soybeans from the market (Endres, 2005).

Whether or not you favor GMO crops, the above two examples highlight the need for regulations ensuring that products labeled as organic or non-GMO have not been inadvertently contaminated with GMOs. A major part of the controversy is about who should pay for any testing associated with identifying instances of contamination and who is liable should a crop become contaminated.

The classic example of Percy Schmeiser vs Monsanto and the contamination of Schmeiser's fields with Roundup Ready Canola was finally settled out of court in 2008, with Monsanto agreeing to pay to clean up Schmeiser's fields (Hartley, 2008).

But the problem doesn't stop with seeds. Think about this: the European Union has banned the importation of Canadian honey because producers cannot guarantee that it is not contaminated with GM pollen (Smyth *et al*., 2002).

The Seed Label and Seed Certification

To ensure full disclosure regarding the status and composition of a seed lot, most seed laws require specific information to appear on the seed label (USDA NRCS, 2009) (Fig. 12.1). As part of the label, seeds are identified as: (i) breeder seed; (ii) foundation seed;

Fig. 12.1. A typical seed label.

(iii) registered seed; or (iv) certified seed. Each level of certification recognizes a specific degree of genetic purity, seed identity, and a minimum level of quality.

Breeder seed is seed that is produced and directly controlled by the breeder and its sole purpose is for generating seeds of the other certified classes. Foundation and registered seeds are seeds directly produced from breeder or foundation seed. Certified seed is produced from breeder, foundation or registered seed. Certification classes are often identified by tag color.

Most growers purchase cultivars of either registered or certified seed. A fairly new type of seed is becoming available called 'Per-Varietal Germplasm' and is often used with native species. These seeds have not been released as a cultivar, but are ready for adoption by growers without the extensive field testing needed for cultivar certification. These seeds are often called ecotypes since they have most of their original genetic identity and their range of adaptation and performance traits have not been examined.

Besides identifying the type of seed for sale, all seed for sale whether certified or non-certified, usually is labeled with information that includes:

1. The species and/or common name and the cultivar or variety that distinguishes the seeds from other similar kinds. If it is a mixture, each specific component above 5% is listed. If the cultivar or variety is not indicated, the label will state 'variety not indicated' or VNI.
2. The lot number with a defined quantity of seeds that must fall within a defined acceptable range.
3. The location where the seed was produced.
4. The net weight of the contained seeds.
5. The percentage purity indicating the amount of pure seed, inert matter, other crop seed, and weed seeds. The total for these four components must equal 100%.
6. The percentage germination.
7. The percentage of dormant seed.
8. The percentage of hard seed. This is seed that fails to imbibe water during the germination test.
9. The percentage of total viable seed (TVS). This is the total of the percentage germination plus the percentage hard seed plus the percentage dormant seed. It reflects the maximum germination that might be expected.
10. PLS (pure live seed) is the percentage of pure seed × the percentage of TVS. This gives a measure of the amount of PLS in the sample.
11. The germination test date.
12. Any restricted noxious seeds indicating the number of seeds per kilogram and the weed name. Many regions have weeds identified as restricted or prohibited. The number of restricted weed seeds per kilogram is limited while prohibited seeds are not allowed at any level. In fact, most seed tests must explicitly state that no prohibited noxious weeds are present in the seed lot.
13. Any seed treatment, which is usually an inoculant or a pesticide.
14. The name and address of the seed company responsible for the analysis included on the label.
15. Any other information including disclaimers, limited warranties, etc.

Germination

Seeds are an amazingly packaged, pre-programmed plant, ready to grow and flourish if given the

right environment. Upon removal from the mother plant either within a fruit or naked, a seed is usually dry and mature and may possess barriers to growth that we call dormancy. The complex nature of seed dormancy was covered in Chapter 10, this volume. For germination to proceed, dormancy must be eliminated. Once dormancy has been removed from the seed, external factors control germination including water, light, temperature, and sometimes nitrate.

A typical dry, mature seed consists of a desiccation-tolerant sporophyte surrounded by a seed coat, or endosperm and a seed coat, depending on the species. Many of the physiological and physical changes associated with germination have been studied using model systems that include peas (*Pisum sativum*), *Arabidopsis thaliana*, cress (*Lepidium sativum*), and tobacco (*Nicotiana tabacum*). Peas store most of their seed food reserves as proteins and starch within the embryo's cotyledons, having no endosperm when seeds are mature. *A. thaliana* and cress have a thin endosperm layer, and tobacco has a thick endosperm layer surrounding the embryo.

Most dry seeds have a water content of about 10% (Weitbrecht *et al*., 2011). Most of the water in a seed is bound and very little is available for metabolic activities. Even so, seeds are not metabolically quiet during the stage between harvest and sowing, often called after-ripening (dry storage of mature seeds at room temperature). After-ripening is characterized by a general improvement in germination percentage and rate with the relaxation of specific temperature, light, or nitrate requirements normally needed to stimulate germination. Even though the process occurs in dry seed, a minimum moisture content, albeit extremely low, is required and is species dependent. Small pockets of free water may exist in many seeds to allow for RNA transcription in specific tissues or organs during after-ripening (Weitbrecht *et al*., 2011). Oily seeds have a lower minimum moisture threshold for after-ripening than starchy seeds. After-ripening will not occur in very high humidity storage.

The exact mechanisms of after-ripening are not known (Finch-Savage and Leubner-Metzger, 2006). During after-ripening, reactive oxygen species (ROS) are formed, accumulate and oxidize proteins. Glutathione works as an antioxidant to protect the seed from ROS oxidation during after-ripening and tocopherol protects membrane lipids from oxidation by ROS during after-ripening. Ascorbic acid seems to play only a very minor role in ROS protection of seeds. It has been proposed that oxidation by ROS early during after-ripening leads to progression through after-ripening and a loss of dormancy, but later may lead to oxidative damage and loss of viability (Bailly, 2004; Oracz *et al*., 2007, 2009). Dry seeds contain abundant mRNAs that survive desiccation and play a role in the metabolic passage through after-ripening and the preparation for and initiation of the metabolism of germination itself.

Factors affecting germination – water

Dry seeds may persist for many years. When planted in the soil or some other growing medium with sufficient water content, germination begins with the imbibition of water. The extremely low water potential of dry seeds leads to a rapid initial uptake of water called phase I that is present in both living and dead seeds. It is a purely physical absorption of water due to water potential gradients. The seed rapidly swells and may change size and shape. There is often leakage of a large quantity of solutes, caused by damage to membranes during rapid rehydration. Various metabolic processes are initiated to repair this damage. Over time the activity of these repair mechansims is reduced and seed viability decreases.

When water uptake in phase I declines and changes in seed size and shape are completed, the seed enters phase II of water uptake. In phase II, water content remains stable as inbibition has reached a plateau. During phase II the embryo begins to elongate. Some species such as *Arabidopsis, Lepidium* and *Nicotiana* possess what is called 'two-step germination': the seed coat (testa) ruptures followed by rupture of the endosperm. Other species without an endosperm such as *Pisum* exhibit 'one-stage germination' where only a testa requires rupturing. Both types of germinating seeds exit phase II and enter phase III, a period of increased water uptake, following the testa rupture. Phase III includes endosperm rupture in two-step germinators and radicle protrusion in both two- and one-step germinators.

Membrane proteins called aquaporins transport water and small molecules across membranes and help cell-to-cell water and solute movement in seeds. They may help regulate the distribution of water within a germinating seed as well as testa rupture. Testa rupture in species where it has been

studied begins at the micropyle. An increase in seed size due to cell expansion and not cell division helps create pressure on the testa leading to its rupture. In species in which it has been studied, cell division does not occur in the embryo during germination. Completion of germination is marked by the protrusion of the radicle through the seed-covering layers (Weitbrecht *et al*., 2011).

Factors affecting germination – light

Germination is often regulated by light via the pigment phytochrome. Exposure to red light after imbibition leads to the formation of P_{fr} which triggers metabolic processes in the seed that facilitate embryo growth. Seed must be imbibing water to sense the light exposure; dry seeds do not respond. If exposure to red light is immediately followed by exposure to far-red light, germination will not occur. Alternately exposing seeds to red and far-red light always results in a response linked with exposure to the most recent light exposure: red light causes germination, far-red prevents it. This is a classic physiology experiment using lettuce seed. Seed and seedling responses to light were covered in Chapter 8, this volume.

Other species in which germination requires exposure to light include *Ageratum, Begonia, Browallia, Impatiens*, and *Petunia*. These seeds should be sown on the surface of the germinating medium and only lightly covered. Seed packets or catalogs provide light requirements for germination.

Depth of planting in the soil and existing canopy cover can greatly influence germination in light-sensitive species via phytochrome mediation (Botto *et al*., 1996; Shinomura *et al*., 1996). Recall from Chapter 8, this volume, the three different types of phytochrome-mediated plant responses based on light levels: (i) the very low fluence response (VLFR); (ii) the low fluence response (LFR); and (iii) the high irradiance response (HIR) (Furuya and Schäfer, 1996). Both VLFR and LFR can be triggered by pulses of light lasting only seconds while the HIR requires much longer exposure, usually several hours. LFR reactions are photoreversible with far-red light and controlled mainly by phytochrome B while VLFR reactions are not and are regulated primarily by phytochrome A. HIR reactions are not photoreversible.

The germination of most light-sensitive seeds is via a LFR-type reaction with exposure to red light. This is the type of response that allows seeds to detect their depth in the soil or their position under an existing canopy and whether they are in the shade or light. For *Arabidopsis* and possibly many other species, this LFR reaction is mediated by phytochrome B and phytochrome E (Hennig *et al*., 2002).

Seeds of many species that are imbibed in the dark often remain photo-dormant and acquire a VLFR phytochrome response mechanism controlled by phytochrome A. These seeds are extremely sensitive to red light and germinate with only a few seconds of very low levels of red light (Smith, 1982). Many weed species utilize this mechanism. When imbibed seeds are deep in the soil, they remain dormant. When disturbed via cultivation and exposure to red light, even only briefly, they resume germination. Remember, germination starts with imbibition, thus even though the seeds are photo-dormant before exposure to red light, they are in the process of germinating. This is a good example of the complexity of the process and how dormancy and germination often overlap in many cases. They are not discrete, separate processes.

Not all seeds germinate in the light. In seeds that can germinate in the dark, exposure to far-red light for long periods inhibits germination via an HIR reaction mediated by phytochrome A. This is the phytochrome response observed in seeds underneath a dense canopy of leaves. The canopy absorbs much of the red light in incoming solar radiation, thus seeds on the ground or buried slightly received a prolonged exposure to far-red-rich light (Smith and Whitelam, 1990). The blue-light-mediated HIR reaction controlled by phytochrome A may or may not have a function in germination.

Factors affecting germination – temperature

Temperature can have a profound effect on both the rate and the percentage of germination by affecting at least three different physiological processes (Roberts, 1988). The first mechanism related to seed germination significantly affected by temperature is seed longevity. As seeds age, their viability decreases and the rate of deterioration is mostly affected by moisture content and temperature. Each species has a different genetically predetermined limit to viability that no alteration of storage conditions can change. In general, the Q10 for the rate of seed deterioration or loss of the ability to germinate is around 2 at –10°C increasing to 10 at 70°C

(Roberts, 1988). Storage of seeds at a low temperature generally minimizes the loss of viability within genetically predetermined constraints.

A second mechanism of temperature control of germination is temperature's effects on dormancy release. The Q10 for loss of dormancy in dry seeds is relatively constant at 2.5–3.8 over a wide range of temperatures up to 55°C. Hydrated seeds are different. Higher temperatures often prolong dormancy or induce a secondary dormancy. Lower temperatures, especially in the range from –1°C to 10°C often remove dormancy through the process of stratification. Low temperatures only infrequently induce a secondary dormancy in imbibed seeds. Alternating temperatures generally remove dormancy and promote germination in imbibed seeds. The level of dormancy release is related to: (i) amplitude; (ii) mean temperature; (iii) the thermoperiod; and (iv) the number of cycles (Thompson *et al*., 1977).

The third way temperature influences germination is through effects on the rate of germination. The percentage germination is primarily determined by the first two mechanisms. In general, once dormancy and any imposed secondary dormancy have been removed the rate of germination is positively related to increase in temperature between the base temperature and the optimum temperature. Germination will not occur below the base temperature and reaches a maximum rate (minimum number of days to germinate) at the optimum temperature. Above the optimum up to the temperature at which germination will not occur (often called the ceiling), the rate of germination is negatively related to temperature.

Most species have an optimum temperature for seed germination and that temperature is often related to climatic adaptation. Those species adapted to cool climates germinate at lower temperatures while those adapted to warmer climates germinate at higher temperatures. Some of the regulation of germination by temperature may be tied to phytochrome, particularly in light-sensitive species. Different forms of phytochrome are more prevalent at different temperatures (Heschel *et al*., 2008). This area of phytochrome–temperature–germination interaction is relatively new and growing (Franklin, 2009).

Factors affecting germination – nitrate

Nitrate (NO_3^-) stimulates germination in many dormant seeds. One hypothesis for nitrate's involvement in seed germination is as follows. Nitrate is reduced to nitrite or hydroxylamine and nitric oxide may be produced from nitrite. All three substances inhibit the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen by the enzyme catalase. The inhibition of catalase leaves some hydrogen peroxide available for peroxidase activity. Peroxidase is responsible for catalyzing the oxidation (removal of electrons) by hydrogen peroxide of many substrates in living cells. Some seeds, such as barley and rice, show a shift from the Embden-Meyerhof-Parnas pathway of glucose utilization (primarily generation of ATP through glycolysis) to the pentose phosphate pathway (generation of NADPH and five-carbon sugars) as they break dormancy and begin to germinate (Hendricks and Taylorson, 1974). The pentose phosphate pathway uses $NADP^+$ as an oxidant (electron remover) which may be limited by the rate of regeneration of $NADP^+$ by reoxidation of NADPH. Nitrate and its reduction products may regulate this reoxidation process.

More recently it has been suggested that nitrate reduces seed dormancy by reducing abscisic acid (ABA) levels (Matakiadis *et al*., 2009). Nitrate reduces ABA levels in imbibed seeds when nitrate is included in the germination medium and also results in lower ABA levels in dry seeds of plants supplied with nitrate during seed development (Alboresi *et al*., 2005). A specific gene (CYP707A2) controlling seed ABA levels that responds to exogenous nitrate has been identified in *Arabidopsis* (Matakiadis *et al*., 2009). In addition, nitrate can initiate production of nitric oxide (NO), a potent signaling molecule (Bethke *et al*., 2006). Again, the complex interaction of dormancy and germination are exposed here. Nitrate appears to relieve dormancy and stimulate germination through effects on ABA and carbohydrate metabolism.

Hormones and germination

ABA induces and maintains the dormant state in most seeds (Kucera *et al*., 2005). During germination the endogenous level of ABA of both dormant and non-dormant seeds declines rapidly during imbibition (Weitbrecht *et al*., 2011). Overexpression of the genes responsible for ABA synthesis increases seed ABA content and enhances dormancy while delaying germination (Lindgren *et al*., 2003; Nambara and Marion-Poll, 2003). ABA-deficient mutants exhibit vivipary (Koornneef and Karssen, 1994; Thompson *et al*., 2000; White *et al*., 2000)

providing further evidence that ABA inhibits germination. ABA inhibits germination at least in part by preventing the transition from phase II to phase III water uptake which then prevents both testa and endosperm rupture as well as radicle emergence (Kucera, *et al*., 2005; Muller *et al*., 2006). When either ABA synthesis or seed sensitivity to ABA are reduced experimentally, germination is enhanced (Kucera *et al*., 2005).

Jasmonic acid decreases and indole acetic acid (IAA) increases during *Arabidopsis* germination (Preston *et al*., 2009). Nitrate seems to regulate both gibberellin (GA) and ABA metabolism in germinating seeds (Alboresi *et al*., 2005) and accelerates the reduction in seed ABA levels during imbibition (Ali-Rachedi *et al*., 2004).

While ABA induces dormancy and inhibits germination, GA promotes germination and counters the effects of ABA. GA has two specific roles during germination: (i) it increases the growth potential of the embryo, which promotes embryo elongation; and (ii) it weakens the tissues surrounding the radicle thereby removing the mechanical constraint to radicle protrusion (Kucera *et al*., 2005). During seed development a large store of inactive GA or precursors accumulate and germination proceeds according to the balance of ABA:GA (active forms). ABA inhibits GA biosynthesis in early germination. Both red light and cold stratification induce GA production and break dormancy in *Arabidopsis* and lettuce (*Lactuca sativa*) (Kucera *et al*., 2005). GA levels are relatively high in dry, mature seeds and biosynthesis of GA is localized in the radicle, hypocotyls, and micropylar endosperm during later germination (Ogawa *et al*., 2003). GA also promotes vivipary (White *et al*., 2000).

Ethylene promotes germination and counteracts ABA's inhibitory effects in many species (Kucera *et al*., 2005; Holdsworth *et al*., 2008; Linkies *et al*., 2009; North *et al*., 2010). Increased ethylene evolution during germination is often observed with many seeds (Matilla, 2000) and a peak of ethylene evolution is often observed as germination is completed. Germination is inhibited if seeds are treated with ethylene biosynthesis inhibitors (Sisler and Serek, 2003; Kucera *et al*., 2005). It is likely that ethylene promotes germination by promoting radial cell expansion of the hypocotyl, increasing seed respiration, increasing embryo water potential, and promoting endosperm rupture (in seeds with two stage germination) (Leubner-Metzger *et al*., 1998; Kucera *et al*., 2005).

Brassinosteroids (BR) work with GA in promoting cell elongation and germination and in counteracting the inhibitory effects of ABA (Kucera *et al*., 2005). BR may act by stimulating GA and ethylene biosynthesis, but this hypothesis is still in question. Cytokinins are abundant in developing seed and seem to play a major role in promoting cell division and development in the embryo and enhancing sink strength of fruit (Kucera *et al*., 2005). While much is known about the involvement of auxin in the first stages of embryo development, information describing the role of auxins in germination is sparse.

Nitric oxide (NO) is a potent signaling molecule in plants (Šírováa *et al*., 2011) so it is no surprise that it is implicated in regulating germination (Zhenga *et al*., 2009). Treating wheat seed with NO by imbibing them in a solution producing NO led to increased germination rate, increased coleoptile and radicle weight, and enhanced respiration and ATP production. NO also increased anti-ROS enzymes, helping protect germinating seeds from the deleterious effects of excessive ROS such as hydrogen peroxide and superoxide anions.

ROS also play a regulatory role in seed germination. Excessive levels of ROS in seeds lead to oxidative damage in cells and a decrease in seed viability. However, cellular signaling relies on at least a minimal level of ROS. The state in between a minimal and an excessive level is called the oxidative window for seed germination (Bailly *et al*., 2008), the state in which germination will proceed.

While specific germination responses to plant hormones and signaling molecules have been described here, keep in mind that germination is regulated by the complex interaction of these compounds. In addition, while much is known regarding hormonal regulation of dormancy and germination, much remains to be understood regarding this incredibly important process.

Metabolism of germination

Metabolism is reactivated with water imbibition during phases I and II of germination. A great deal of work has been performed to determine whether the increase in metabolism associated with germination is due to newly formed enzymes or due to enzymes stored in the seed. In *Arabidopsis*, translation of mRNA is required for germination while inhibition of transcription of DNA to RNA does not inhibit germination (Rajjou *et al*., 2004). Tobacco (*N. tabacum*), however, proceeds to testa

rupture but requires transcription to complete endosperm rupture (Leubner-Metzger, 2003). Thus it appears that the initial metabolism activated by water imbibition during germination is via stored enzymes or their mRNA, but completion of germination may require newly synthesized enzymes.

Upon imbibition, a sharp increase in oxygen uptake and carbon dioxide evolution by the seed is observed, but then quickly stagnates until the end of phase II (Bewley, 1997). A sharp increase in ATP production is concomitant with imbibition (Benamar *et al*., 2008) and levels increase as mitochondria are repaired or synthesized during the early stages of germination. Stored sugars, lipids, and proteins provide substrates for energy production.

Some practical implications for seed dormancy and germination

Dormancy and germination are intimately linked and the practical implications for such an interaction are worth discussing. In many crops, a certain level of seed dormancy at harvest is desired to prevent viviparous germination either while on the parent plant or after harvest. However, for some crops, such as those used for sprouting or where germination is crucial to their economic use (malted barley, *Hordeum vulgare*, for example), dormancy is not desirable. When seeds are used for propagation rather than food or feed, dormancy is not desirable as rapid and uniform germination are necessary elements of productive culture. As long as environmental factors such as light, temperature, and moisture can be carefully regulated during storage, dormancy is not needed.

Since many seeds exhibit dormancy after harvest, a number of seed treatments have been developed to improve germination upon planting. Treatments include: (i) a period of after-ripening; (ii) stratification; (iii) heat treatment to simulate fire; (iv) scarification; (v) hormone treatments; and (vi) priming. Each species may have specific treatments to enhance removal of dormancy and/or enhance germination. Many of the treatments that improve germination may also reduce storability.

As novel methods of weed control are developed for low-input operations, better understanding of the complex nature of weed seed dormancy and germination integration is imperative. Many weed species cycle in and out of dormancy allowing them to remain viable for years in the soil, while at the same time, being ready to germinate under specific environmental conditions. This weed seed bank in the soil is often difficult to deal with in many weed control strategies.

Seeding and factors affecting establishment

With an understanding of the basic physiology behind seed dormancy and germination, now consider the physical act of planting seed for horticultural production. While many seeds are sown directly into the production field, there are also many crops that are started in the greenhouse and transplanted to the production field. We will examine both approaches.

Greenhouse seeding

Seedlings started in the greenhouse may be directly sown into the cell trays in which they will grow while in the greenhouse or they may be sown in a germinating flat and transplanted into production trays a week or two after germinating. The first approach requires seed large enough for singulation. The second approach requires labor skilled enough for transplanting seedlings. Transplanting seedlings also subjects seedlings to the stress of a disturbed root system. With either approach, appropriate containers and an appropriate medium are needed.

CONTAINERS Most commercial containers for seedling production in the greenhouse are plastic, Styrofoam®, or a sustainable material such as wood, compressed fiber, or peat moss. Some greenhouse operators use homemade soil blocks and avoid the use of a container altogether. Soil blocks are labor intensive and are mostly used in smaller operations.

Styrofoam® and plastic are the most widely used trays with plastic being preferred over Styrofoam®. This is because: (i) Styrofoam® trays are generally more expensive than plastic; (ii) they insulate the root systems of seedlings leading to slower growth; and (iii) they may also promote algal growth and disease development. In addition, Styrofoam® trays cannot be nested like plastic inserts for storage. Plastic seedling trays normally consist of two parts:

1. An outer tray measuring 28 × 53 cm (11 × 21 in), also called the standard 1020 tray, with or without holes for drainage. Trays with drainage holes are usually recommended.

2. The second component is called an insert, which is normally placed inside the tray to increase sturdiness. Inserts can be used without the outer support tray, however this is not recommended. The inserts are identified by a number indicating the number of plugs per insert or standard 1020 tray.

Standard inserts are available in 24, 36, 38, 50, 72, 98, 128, 200, 288, 406, 512, 600, and 800 sizes (Fig. 12.2). Individual cells range from 2.5 to 6.3 cm (1–2.5 in) deep and are either round or square. Other sizes are available for special needs. Inserts from 24 to 72 are often called finishing or transplant trays. Higher count inserts (98–800) are often called plug trays. Plants germinated in plug trays must be transplanted into finishing trays.

Many growers opt to purchase plugs and finish them off in their own greenhouse after transplanting to larger cell sizes, typically in the 24–72 cell size. Specialty nurseries grow seedlings in the 400–800 tray size for just this market. Other growers prefer growing their own seedlings. With this approach, they don't have to rely on the cultivars available from commercial plug producers. They can direct seed into production trays or transplant young seedlings from germination flats (standard 1020 trays can be used as germination trays) or their own plug trays. There are a wide variety of devices available for seeding greenhouse trays and their costs vary considerably. With machine-seeded flats, flat sizes may be 400, 500, 600, or 800 cells per flat. Many smaller growers sow seed in plug trays by hand or using hand-held devices.

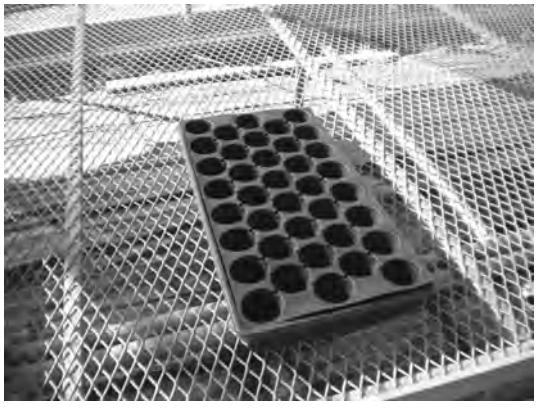


Fig. 12.2. A standard 38-cell insert for greenhouse seeding or transplanting.

Selection of finishing tray size is based on: (i) the species; (ii) the number of seedlings required; and (iii) the greenhouse space available for production. Larger cells are much less likely to suffer from stress-induced premature flower formation. Even though retail sales may prefer smaller transplants with flowers, ultimate field performance is best with transplants that have not been prematurely induced to flower. This is true for both flowering annuals and vegetable transplants. Thus, it is best to go with the largest cell size that can be accommodated in the available space.

Larger cells result in faster maturing seedlings but take up more room per seedling in the greenhouse and are therefore more expensive to produce. There is no evidence that cell size influences final yield as long as cells are well watered. Smaller cells dry out more readily than larger cells, thus are more prone to stress should watering become compromised.

Organic growers may benefit from larger cell sizes due to greater nutrient availability per seedling in larger cells. Organic transplant production is complicated by the lack of a high-nitrogen water-soluble fertilizer for application during the later stages of greenhouse production when nutrient supply from the growing medium has been exhausted. Fish emulsion is the classic fertilizer used in organic transplant production. It is relatively low in nitrogen (about 5%) and often has an unpleasant odor. In non-organic seedling production, there are many high-nitrogen, water-soluble fertilizers available for use.

MEDIA There are many growing media available for use in greenhouse transplant production and a description here of all of them would not be possible. However, a good greenhouse medium should posses certain attributes pertaining to: (i) drainage; (ii) chemical properties such as pH and soluble salts content; (iii) sterility; and (iv) nutrient availability.

Greenhouse media must be well drained. Larger particle sizes provide better drainage, but require more frequent watering. Additionally, smaller particles facilitate easier plug cell filling. Poorer drainage of smaller particle sizes can be accounted for by less frequent watering.

A good greenhouse medium should have a pH of 5.5–6.5, and have a balanced, low level of nutrients. Excess nutrients can lead to succulent, tender growth that is difficult to harden off before field transplanting. Soluble salts should be in an acceptable range indicated by electrical conductivity values

between 0.4 and 0.6 μS/cm for an extract made by mixing one part soil with two parts distilled water.

Greenhouse media should be free of plant pathogens but does not necessarily have to be sterile. Most soil-less mixes are purchased pre-sterilized or their components are purchased before mixing on site.

The base components of a good growing medium include several of the following: peat moss, perlite, vermiculite, coconut coir, rice hulls, screened bark, compost, and sterilized soil. Peat moss, coir or compost normally provide the bulk of the medium, and perlite, vermiculite, bark or rice hulls are added for drainage. There are many recipes for seedling media available online. The key to success is to use the highest quality materials available and mix the media carefully, paying close attention to pH of the final mix and measure lime and fertilizer amendments carefully.

In many regions, commercially prepared mixes are available that are quite affordable and often do not cost much more than the separate components used to make a mix on site (Fig. 12.3). Mixes are also now widely available that conform to organic production regulations. Organic mixes made on site should contain significant amounts of good quality compost if possible to ensure adequate nutrition of seedlings.

Fig. 12.3. A bale of standard potting medium for greenhouse production of transplants. Most commercial media are a mixture of ingredients which may include peat moss, pine bark, coir, rice hulls, vermiculite, perlite, lime, starter nutrients, a wetting agent, and other proprietary ingredients.

FERTILIZING SEEDLINGS Most greenhouse seedlings require some nutrients to supplement those available from the growing medium. The quality and field productivity of spring-grown greenhouse transplants is linearly related to fertility levels (Vavrina *et al.*, 1998). In non-organic production systems, this is easily accomplished with a balanced water-soluble fertilizer. Many formulations are available from greenhouse supply firms. Organic production systems are more problematic. Nitrates are the nutrient most needed by greenhouse seedlings (Van Iersel *et al.*, 1998), and most organic nitrogen sources provide slowly available, low levels of nitrates to seedlings. In addition, most organic sources of nitrogen do not supply nitrate directly. The nitrogen is normally in a more complex form, such a protein, which must be broken down into ammonium (mineralization) then converted to nitrate by bacteria in the medium (nitrification). Ammonium can be taken up and used directly by plants, however, most nitrogen is utilized as nitrate. Too much ammonium under low light or cool soil conditions (<12°C) can be toxic to seedlings.

Most organically produced transplants are fertilized with fish emulsion fertilizer or compost tea on a fairly frequent basis soon after seedling emergence. Fertilizing with a low level of nitrogen at every watering is possible. However, to avoid over fertilizing, every week or every other week is often more suitable.

WATERING SEEDLINGS The key to watering greenhouse-grown seedlings is to prevent drying out and water stress while at the same time avoiding overwatering, which is also stressful and may lead to root rot and epinasty. Common sense and observation are the best tools. A good rule of thumb is to water an hour or two after sunrise and then check the greenhouse again just after noon, especially on warm sunny days. Avoid watering late in the day to avoid night-time humidity problems and increased disease potential. The growing medium should be allowed to dry nearly to the point of wilting before rewatering. Apply enough water to thoroughly wet the medium, but do not overwater. Just because

water flows from container drainage holes does not mean that the medium has been thoroughly wetted. If the growing medium dries out too much, it often pulls away from the side of the container and water flows around the root ball and out of the container rather than through it.

When making watering decisions consider: (i) the seedlings' stage of production; (ii) the greenhouse temperature; and (iii) the current weather conditions outside (is it sunny or cloudy?). Pay very close attention to germination flats as they are easy to overwater. But never let germination flats or seedling flats dry out completely either.

HARDENING OFF TRANSPLANTS Before setting in the field, transplants are gradually conditioned to tolerate harsher conditions found in the field as compared with the greenhouse. Hardening off is usually accomplished over a 1–2 week period. It consists of increasing daily exposure to field conditions which includes: (i) higher direct sunlight; (ii) less frequent watering; and (iii) exposure to wind. Night-time temperatures should be considered when hardening off sensitive crops.

There are various approaches used to accomplish the same goal. Transplants are often moved outside to a semi-protected area for several hours a day for a few days to acclimate them to outdoor conditions. The daily length of exposure is gradually increased and protection gradually removed such that by the end of the second week, transplants have spent several days and nights outside, under conditions similar to the production field.

Moving transplants in and out of the greenhouse during the first stages of hardening is labor intensive. Therefore, hardening may be accomplished in an area outside the greenhouse that is protected from the direct outside environment with some sort of shade cloth or in a lath house. Gradually the protection is removed.

TEMPORARY STORAGE OF TRANSPLANTS Sometimes field planting of transplants must be delayed due to circumstances beyond the control of the grower such as inclement weather. Additional fertilization is often ineffective in maintaining slow growth of seedlings because root volume has been filled. If held in the greenhouse, such transplants are often stressed to the point that they flower prematurely thereby greatly reducing productivity in the field. Flats of such seedlings can be temporarily held in a state of 'suspended animation' allowing a grower more time to prepare the production field.

The temperature and length of storage depends on the species being held (Gast and Stevens, 1994). All species store better with exposure to light of at least 5 foot candles when stored in cold-storage facilities (Fig. 12.4). Supplemental light is not

Fig. 12.4. Plug plants held in a cooler until suitable conditions for planting occur.

needed when stored in cold frames or cool greenhouses. *Botrytis* can become a major problem in stored plugs, especially with lushly growing plugs. After storage, plugs should be slowly warmed to 15–20°C (60–70°F) under low light conditions before transplanting.

Since most growers do not have the luxury of access to many different temperatures for temporary storage of plugs, some general recommendations were made by Gast and Stevens (1994) and are listed in Table 12.1.

Field seeding

Many crops are directly seeded in the production field rather than being transplanted from greenhouse-grown seedlings. Field-seeded crops do not experience the luxury of a controlled environment during germination which is the most crucial event during stand establishment. In order to maximize germination success, proper seed bed preparation, seed spacing, depth of soil coverage, and irrigation following planting are crucial.

SEED BED PREPARATION The seed bed should be well tilled before sowing unless no-till seeding is practiced. The main mistake made during seed bed preparation is over preparing the soil for sowing. The idea that a seed bed must be prepared to create good seed-to-soil contact is somewhat misleading. While it is true that good seed-to-soil contact allows capillary water in the soil to flow towards the seed and surround it, most of the water imbibed by seeds is in a vapor form. Only about 10% of a seed's surface is in contact with liquid water during imbibition in soil that is near field capacity. It is more important to ensure that soil moisture losses are minimized during germination by covering the seeds adequately with either soil, mulch or both.

Excess tilling leads to extremely fine soil which is likely to form a hard, compact crust when the soil dries following post-planting irrigation. While this crust does reduce water loss from the soil, the force required to break this crust is often much greater than a germinating seedling can generate. Seedling emergence is then severely compromised.

DEPTH OF PLANTING Some seeds such as lettuce (*L. sativa*), require light for germination and should be sown shallowly. Light-regulated germination is most often controlled by phytochrome. The seed must be covered with enough soil to create a microclimate that supports water imbibition but not so much soil that light penetration is severely decreased. When seeds are covered with enough soil to promote imbibition, enough red light can penetrate the layer to induce the formation of P_{fr} and signal the commencement of germination.

Depth of seeding must also consider seed size and the ability of the seed to supply energy during germination via reserves until emergence and the inception of photosynthesis. If planted too deeply, a seed may exhaust all stored food before it can emerge from the soil and photosynthesize. However, if seeds are not planted deeply enough, poor seedling anchorage will result leading to poor stand establishment.

SPACING In-row seed spacing should be based on final plant densities required to optimize yield as well as whether or not seedlings will be thinned. Seeding density must also consider germination percentage. Seed lots with low germination should be seeded more densely to compensate for reduced germination and thinned if needed.

Between-row spacing is often determined by equipment requirements. In situations where hand labor is mostly utilized, between-row spacing is determined by optimum plant densities and management requirements. In both instances, spacing should utilize the land area to: (i) optimize yield; (ii) ease management; and (iii) protect the soil from wind and water erosion.

METHOD OF SEEDING Seed can be sown by hand or by using various types of seeders that are available from a number of sources. Even in small operations, the use of mechanized seeding can save time as well as reduce seed costs by more efficient and even placement of seeds during sowing.

Seed technology

Many crops are mechanically planted with precision seeders to: (i) reduce the number of seeds used per acre; (ii) reduce labor requirements for seeding; and (iii) reduce seedling thinning after germination. Many seed technologies have been developed to make precision planting easier, enhance germination, improve stands after germination, and improve uniformity at harvest. Seed technologies include: (i) mechanical seed treatments; (ii) pelleted seed; (iii) seed encrusting; (iv) film coating; (v) seed priming; (vi) seed-lot upgrading; (vii) artificial seeds; and (viii) genetic use restriction technology (GURT).

Table 12.1. Recommended temperatures and storage times (in dark and light) for plugs and seedlings of various flowering annuals and annual vegetables (after Gast and Stevens, 1994).

Species	Cultivar	Optimum storage temperature (°C)	Maximum storage time (weeks)	
			In the dark	With light[a]
Ageratum	'Blue Danube'	7.2	6	6
Alyssum	'New Carpet of Snow'	7.2	–[b]	3
	'New Carpet of Snow'	2.5	5	6
Fibrous begonia	'Viva'	7.2	–	1
	'Vodka'	5.0	6	6
Tuberous begonia	'Nonstop Scarlet'	7.2	–	6
	'Nonstop Scarlet'	5.0	3	6
Celosia	'Cherry Red'	7.2	–	1
	'Cherry Red'	10.0	2	3
Coleus (*Solenostemon*)	'Multicolor Rainbow'	7.2	–	2
Cyclamen	'Giselle', 'Sylvia'	7.2	–	6
Dahlia	'Amore', 'Figaro'	7.2	–	6
	'Amore', 'Figaro'	5.0	2	5
Geranium (*Pelargonium*)	'Pinto Red'	7.2	–	4
	'Pinto Red'	3.0	4	4
Impatiens	'Accent Lilac', 'Accent Red Star', 'Super Elfin Orange'	7.2	–	2
	'Accent Orange'	7.5	6	6
Lobelia	'Blue Moon'	7.2	–	5
	'Blue Moon'	5.0	6	6
Marigolds (*Tagetes*)	'Scarlet Sophia'	7.2	–	5
	'Hero Yellow'	5.0	3	6
New Guinea Impatiens (*Impatiens* × *hawkeri*)	'Kientzler Series', 'Paradise Series'	12.5	2	3
Pansy (*Viola*)	'Majestic Yellow'	7.2	–	6
	'Majestic Yellow'	2.5	6	6
Pepper (*Capsicum*)	'Better Bell'	7.2	–	2
Petunia	'White Flash'	7.2	–	5
	'Ultra Red'	3.0	6	6
Portulaca	'Double Mix'	7.2	–	5
	'Fuschia'	7.5	5	5
Salvia	'Red Hot Sally', 'Red Pillar'	7.2	–	4
	'Red Hot Sally'	5.0	6	6
Tomatoes (*Solanum lycopersicum*)	'Better Boy'	7.2	–	4
	'Rutgers'	7.5	3	3
Verbena	'Showtime Mix'	7.2	–	4
	'Romance Mix'	7.5	1	1
Vinca	'Bright Eyes', 'Grape Cooler', 'Little Linda', 'Little Pinkie', 'Polka Dot', 'Pretty 'n' Rose'	7.2	–	3
	'Peppermint Cooler'	10.0	5	6

[a] At least 5 foot candles of light.
[b] –, Indicates this has not been tested at this temperature in the dark.

Mechanical seed treatments

Mechanical seed treatments are treatments that improve a seed's reproductive quality by: (i) scarring or removing the seed coat; (ii) removing the hardened pericarp; or (iii) sorting into defined sizes or densities. All of these treatments improve water imbibition or facilitate mechanical seed planting.

As an example, consider the combination of several mechanical technologies used in sugarbeet seed production (*Beta vulgaris*). Beet seeds are really fruit (schizocarps) where the dried, hardened pericarp surrounds one or more seeds with rather rough projections from the globular fruit. In order to facilitate precise mechanical planting, these projections are removed by polishing the seed. A second step sorts the polished seeds into different sizes, allowing precise placement in the field. A third technology, monogerm cultivars, may be employed; these contain only one seed per fruit, thus eliminating the need for post-emergence thinning.

Pelleted seed

Seed pelleting adds a coating of an inert material such as clay to irregularly shaped or very small seeds (Fig. 12.5). By transforming irregularly shaped or long seeds into round, easily singularized seeds, precise mechanical planting can be achieved (Fig. 12.6).

Improvements have been made in pelleting seeds to increase oxygen permeability at planting, including pellets which split open upon hydration. Different coating materials can vary pellet density for increased precision. Heavier pellets tend to drop quickly from the metering device to the soil with little pellet bounce in the furrow allowing faster tractor speeds during planting. Seed protectants are often incorporated into the pellet. Protectants applied in this manner reduce the total amount of chemical applied per hectare and is very target specific.

Fig. 12.5. Pelleted, primed lettuce (*Lactuca sativa*) (left) seed compared with non-pelleted seed (right).

Seed encrusting

Encrusting adds weight to a normally lightweight seed to enable more precise mechanical planting by encasing the seed in an inert material. The overall seed shape may or may not be modified. Many sweet corn cultivars have light-weight, irregularly shaped seeds that are difficult to mechanically plant. Encrusting adds weight and improves their shape for handling by mechanical planters. Encrusting is less expensive than pelleting.

Film coating

Some seeds, such as those of the *Brassica*, are large and regularly shaped and do not require pelleting. In these cases, protective chemicals can be applied in a film of synthetic polymer. Polymers have the advantage of producing a thin coat of tightly adhered protectant, preventing loss of the material while adding little to the weight of the seeds. Additionally, reduced worker exposure to the chemical and its dust improves worker safety.

A rather novel use of thin polymer coating is the use of temperature-sensitive water-permeable films which only allow imbibition once the soil temperature has reached a certain minimum. This prevents germination before soil environmental conditions are favorable for continued seedling growth.

Seed priming

An increasingly common practice in horticultural production is seed priming. Seed priming consists of precisely controlled water imbibition allowing germination to begin. At a precisely defined stage before radicle emergence and before desiccation tolerance is lost, germination is halted and the seed is dried for later planting. Once planted, germination is completed with radicle emergence.

Since many of the physiological processes of germination are well under way in primed seeds, the metabolic phase of germination is greatly shortened, thus germination time and time to emergence in the field is reduced by up to 50% compared with non-primed seeds. Additionally, priming increases the effective temperature range for germination, thereby allowing germination at less than optimal temperatures. Priming also promotes the proliferation of root hairs, which may lead to more efficient nutrient and water uptake. In crops such as lettuce and celery, priming releases the seeds from phytochrome-induced

Fig. 12.6. A nice stand of lettuce (*L. sativa*) achieved by mechanical planting of primed and pelleted seed.

dormancy. Priming also improves the final stand of many commercial crops which promotes uniformity at maturity.

A beneficial side effect of priming is the reduction of several seed-borne pathogens, including *Xanthomonas campestris* in *Brassica* seeds and *Septoria* in celery (*Apium graveolens*). The likely causes of this reduced pathogen load is the water potential the organisms are exposed to during priming, sensitivity to priming salts, or oxygen concentration.

A major disadvantage of primed seed is that they have a shorter shelf life and are more expensive than their non-primed counterparts.

Seeds can be primed in several ways and though different, all achieve the same results. The key to priming is slow and controlled imbibition. Most commercial priming methods are proprietary. Osmo-priming (osmo-conditioning) is the standard method for priming seeds. Seeds are soaked in a water solution where water potential is precisely controlled with polyethyleneglycol or potassium chloride. A variation of this method, matrix-priming (matrix-conditioning) includes hydrating seeds in a matrix such as vermiculite or absorbent polymers.

Soaking seeds in warm water overnight is a simple method of hydro-priming. Seeds primed this way are not dried, but rather, planted immediately. Many growers hydro-prime long germinating or difficult seeds, for example carrots (*D. carota*) and beets (*B. vulgaris*), in an attempt to hasten germination. Care must be used as some crops cannot be primed, such as green bean (*Phaseolus vulgaris*).

THE PHYSIOLOGY OF SEED PRIMING The uptake of water during imbibition occurs in three stages. Stage I is imbibition where there is a rapid initial water uptake due to the seed's low water potential. During this phase, proteins are synthesized using existing mRNA and DNA and mitochondria are repaired. In stage II, there is a slow increase in seed water content, and physiological activities associated with germination are initiated, including synthesis of proteins by translation of new mRNAs and synthesis of new mitochondria. Stages I and II are the foundations of successful seed priming where the seed is brought to a seed moisture content that is just short of radicle protrusion. There is a rapid uptake of water in stage III where the process of germination is completed culminating in radicle emergence.

Priming induces rRNA synthesis and the repair of DNA damaged during normal seed ageing. Many enzymes involved in metabolic processes involved in germination are also synthesized or induced. This includes enzymes responsible for mobilization of storage proteins (peptidases), carbohydrates (α and β amylases), and lipids (isocitrate lyase) that are particularly needed after radicle emergence.

Other enzymes that increase during priming include catalase and superoxide dismutase, both free-radical scavengers important in minimizing cell damage. Priming induces osmotic stress in seeds, thus low-molecular-weight heat shock proteins often appear during priming. They may help maintain the proper folding of other proteins, facilitating entry into proteolytic pathways when needed during germination.

The priming process is stopped just prior to radicle emergence so that the seed's desiccation tolerance is not lost. Cell enlargement is responsible for radicle emergence and cell division begins after emergence commences. Cell division is not affected by priming; however, priming does advance the cell cycle up to mitosis, so the seed is 'ready to go' once it is planted. In addition, primed seeds have increased levels of ATP compared with non-primed counterparts and the levels remain high for 4–6 months after drying. Upon planting and imbibition, primed seeds are 'loaded' with ATP energy

compared with non-primed seeds making the primed seeds more vigorous during germination.

Priming can also release seeds from dormancy in certain crops. Many seeds of cultivars of lettuce (*L. sativa*) are thermodormant at high temperatures (30–35°C). The embryos of lettuce seeds are enclosed in an endosperm that is two to four cell layers thick, with cell walls composed primarily of glactomannan polysaccharides through which the radicle must penetrate for germination to be complete. The enzyme endo-β-mannase is the enzyme responsible for endosperm weakening and it requires ethylene for activation. High temperatures inhibit ethylene production and thereby inhibit cell wall loosening and radicle protrusion. Priming somehow improves ethylene production by the embryo, enhancing the activity of the cell-wall-loosening enzyme. Some seeds such as tomato (*Solanum lycopersicum*), carrot (*D. carota*) and cucumber (*Cucumis sativus*) do not require ethylene for germination. Priming enhances loosening of cell walls in these species as well, allowing germination at less than optimal temperatures. Enzymes regulating cell wall loosening are probably activated by the priming process.

Seed-lot upgrading

Viable seeds are often more dense than non-viable ones. By separating seed lots by density, improved germination rates nearing 100% can be obtained by removing the non-viable seeds based on their lower density. Sorted seed can also be primed, improving their performance even more.

Artificial seeds

New technologies in cell culture and regeneration allow large-scale somatic embryo production. Somatic embryos are vegetatively derived clones. The embryos can be placed in a gel-like matrix of agar, gum or dextrans and surrounded by an artificial seed coat. The 'seeds' can then be used for propagation much like normal seeds.

Genetic use restriction technology (GURT)

GURT, commonly known as terminator technology, is a controversial issue in contemporary agriculture. Whether you support or oppose GURT is not germane to this text. Rather, knowledge of the basic mechanisms of GURT will provide the information needed to argue either way intelligently.

The technology was developed by the Agricultural Research Service of the USDA and Delta and Pine Land Company in the 1990s. Monsanto Company, the world's largest seed company pledged not to commercialize this technology in 1999. In 2006, Monsanto bought Delta and Pine Land Company.

GURTs are a range of strategies designed primarily to impede transgene movement. There are two major types of GURTs: (i) T-GURTs (trait-GURTs); and (ii) V-GURTs. T-GURT plants are such that they possess some form of genetic enhancement but the level of expression of the trait is controlled via application of a proprietary chemical treatment. T-GURT plants are fertile, produce viable seed, and the enhanced gene is passed on from generation to generation. The gene's expression is under control of the developing company via its proprietary chemical. If a farmer purchased T-GURT seed from Company X, he or she would also have to purchase the proprietary chemical from Company X to induce the desired trait. The farmer could save seed for planting the next season; however, the desired trait would not be expressed lacking the proprietary chemical.

V-GURT plants prevent transgene movement by producing sterile seeds. If a farmer purchased V-GURT seeds, he or she could not save the seed for the next growing season, since it would produce sterile plants which would not produce fruit or seeds. The farmer would have to purchase new seeds annually. This is no different from the current practice of buying hybrid seed.

GURTs can prevent transmission of transgenes out of their desirable domain, thereby limiting the flow of genes into the wild. GURTs also protect proprietary germplasm. Even though this is one of the more controversial aspects of the issue, it is no different from seed companies protecting their germplasm by developing proprietary hybrids which have been used for years in world agriculture.

13 Pruning, Training, Growth, and Plant Size

Pruning versus Training

It is important to distinguish between pruning and training. Pruning is the removal of tissue from a plant by cutting or pinching. Training is the establishment of a desirable size and shape of a plant to achieve specific goals, such as aesthetics in the landscape or fruiting in an orchard. Well-trained plants are developed through proper pruning. Training also includes directing growth into a desired direction using metal or wooden spreaders, string or rope, weights hung from branches, poles, and trellises.

Pruning is most often performed during the winter while training usually includes dormant and summer pruning as well as growth placement during the growing season. A major goal of training is to direct plant growth to minimize the amount of pruning needed for the desired effect.

Pruning

Pruning is performed for a variety of reasons. It is often a source of great angst for inexperienced horticulturists, but it shouldn't be. Pruning is performed for very specific reasons in an organized and logical manner to achieve some very specific goals. Pruning should be viewed as a planned series of operations rather than a one-time event. If the pruning process is well thought out and planned, great results can be easily achieved.

Many perennials are pruned regularly. Heavy pruning for training in the early years is followed by lighter maintenance pruning later on. Many annual flowers are pruned by pinching to encourage a nicely branched plant and promote bushiness. Senesced flowers are also pruned or deadheaded to encourage continued flowering and prevent seed and fruit set. Some annual vegetable crops, most notably tomatoes (*Solanum lycopersicum*) and trellised cucumbers (*Cucumis sativus*) require pruning to maximize yield and facilitate easy management. Biennial ornamentals such as roses (*Rosa* spp.) must be regularly pruned to promote flowering and reduce disease and insect pressure. Biennial fruit crops such as floricane fruiting raspberries and blackberries (*Rubus* spp.) are also pruned. Most perennials which include both ornamental and fruit crops, require some amount of pruning.

It is far beyond the scope of this text to cover pruning and training of specific horticultural crops. There are many excellent references covering specific pruning recommendations. This chapter will focus on the physiological ramifications of pruning. Understanding what the different cuts of pruning and different positioning effects of training are doing to the plant will make decisions about whether or not to make them much easier.

Reasons for pruning

There are many different reasons for pruning and knowing why you are pruning makes decisions during the process easier to make. Pruning should always consider safety first, especially in ornamental situations. For example, a large dead limb should be removed from a tree before it falls and hits someone on the head. After safety, plant health is considered. All dead or injured branches should be removed with an appropriate-type cut. Branches or limbs that rub together should also be removed.

Pruning is often performed to establish, maintain and adjust the plant for its intended purpose. In the landscape, this often includes creating and maintaining a particular size and shape. Keep in mind that the best approach is to try and follow a plant's natural size and shape when pruning. Most plants usually have either one central dominant shoot with laterals that don't really compete with the main stem (many evergreens) or several co-dominant shoots that are competing with each other (many deciduous species). Don't try to prune a plant into a size and shape it would not naturally assume since in the end it is a losing battle. When pruning is

appropriate for a specific landscape plant it is often difficult to tell that it has even been pruned.

In the orchard or vineyard, pruning is performed for size and shape control as well as to create and maintain a productive fruiting canopy. Fruit crops are often trained and pruned more severely than landscape plants and are often forced to conform to 'unnatural' configurations to facilitate management. Pruning fruit crops often increases fruit quality by improving overall light exposure. Pruning to improve light penetration into the canopy also helps maintain fruitfulness by encouraging flower bud formation well into the canopy rather than just on the periphery. Opening up the canopy also improves the penetration of pest control sprays making them more effective. Pruning is often performed to either stimulate vegetative growth of trees that are particularly low in vigor or to remove excessively vigorous shoots that cause shading. Pruning can also be used to renew fruitfulness of trees that have declined in productivity over time.

Careful pruning of both ornamental and fruit species produces a strong framework. Well-angled branches can be selected early in the plant's life to produce strong main scaffold limbs. Well-selected scaffolds lead to better light penetration into the canopy and increases plant vigor and productivity.

Pruning equipment

Effective pruning requires the right tool. For smaller jobs or fine-detailed pruning, hand tools are used (Fig. 13.1). For larger jobs or those not requiring detailed work, mechanical equipment may be required.

Fig. 13.1. Bypass (left) and anvil (right) pruning shears used for hand pruning.

Shears

Shears are capable of making cuts up to about 2 cm. There are two kinds of hand shears: (i) bypass (also called cross-cutting, scissor, or draw-cut); and (ii) anvil (also called snap-cut). Bypass shears cut by gliding one sharp blade against another thicker blade much like a pair of scissors. Anvil shears make cuts by pressing a sharp blade against a broad flattened anvil. Bypass shears are more desirable because anvil shears tend to crush the stem while cutting.

Loppers

Loppers or lopping shears are like regular shears with larger blades and much longer handles to provide more leverage for making bigger cuts, up to 4 cm. Look for loppers with rubber shock-absorbing bumpers between the blades. Again, use only cross-cutting loppers. Cut with one smooth motion to avoid tearing and injuring the plant. Both shears and loppers are available with ratchet action to provide more leverage for those with arthritis or poor hand strength.

Hedge shears

Hedge shears have long, cross-cutting blades designed for trimming hedges. Blades are normally around 30 cm long with similar length handles. They are used exclusively to prune the small, succulent growth of hedges. Electric and gas-powered hedge trimmers are also available. They normally utilize two blades that oppose each other and slide back and forth over each other to achieve the cutting action desired.

Saws

Pruning saws are often needed for cuts larger than those that can be easily made with loppers or shears. Saws are available in many configurations but typically are either bow saws or blade saws. Bow saws consist of a blade with large, sharp teeth mounted on a metal frame shaped like a slightly misshaped upper case letter 'D'. They come in a variety of sizes, can make large cuts but may be awkward for reaching into tight spaces. Blade saws may be rigid or folding and consist of a narrow blade with coarse teeth attached to a wooden handle that is either D-shaped or crescent-shaped.

Teeth on a blade saw are often finer than those of a bow saw blade. Pruning saw blades are often curved and usually cut on the draw stroke (pulling the blade towards you).

Pole pruners

Pole pruners are used for cutting branches high up in a canopy without using a ladder or other means of height extension. They consist of a saw blade and a lopper attached to a long pole of wood, fibreglass, or aluminum. The lopper is operated by a long rope that is pulled downwards. Be careful when using an aluminium-handled pole pruner around power lines.

Electrical or gas-powered chain saws are used to remove large branches. They should be used with care and never above the shoulders or when the operator is on a ladder.

Tractor-mounted equipment

Loppers attached to tractor-mounted air compressors are often used in orchard settings. These units are loppers with a trigger-activated cutting mechanism. Compressed air provides the cutting power thereby reducing human effort required for large jobs.

There are two types of tractor mounted mechanical hedgers and toppers. One operates as a series of circular saws mounted on to a windmill mechanism, with three or four rotating arms, while the other is a rigid arm with three or four overlapping saw blades. Both work well, but the windmill type can remove some of the cut brush as it rotates.

Disinfecting pruning equipment

It is a good idea to disinfect pruning equipment before and after each day's use. More frequent disinfecting should occur if you are pruning specimens that may be infected with certain disease organisms. Diseases caused by organisms carried in the vascular tissue may be easily spread via contaminated pruning equipment. Thus when dealing with these types of diseases, frequent disinfecting with a household cleaner such as Lysol® or a bleach solution (one part commercial bleach to nine parts water) is recommended. Lysol® is least corrosive to pruning tools while bleach is extremely corrosive (Teviotdale *et al*., 1991). Oil tools regularly to prevent corrosion.

Safety goggles

Safety goggles or glasses should always be worn when pruning, and especially when using pole pruners.

Types of cuts

Effective pruning and training requires a number of different types of cuts, each used when a specific result is desired from the cut.

Pinching

Pinching is the process of manually removing the terminal portion of a succulent shoot in order to promote bushiness (Fig. 13.2). It is most often accomplished without tools and is usually performed on ornamental annuals to create nicely shaped plants.

Heading back cut

A heading back cut involves removing the terminal portion of a shoot or stem back to a bud (Fig. 13.3). The bud should be positioned to provide new growth in the desired direction. If there are two vegetative or mixed buds at a node, remove one of them to induce growth in only one direction. Leaving both of them will result in weak, forked growth. If the pruning

Fig. 13.2. Pinching removes the terminal portion of a succulent shoot in order to promote bushiness.

Fig. 13.3. A heading back cut involves removing the terminal portion of a shoot or stem back to a bud.

Fig. 13.4. A bench cut is a modified heading back cut. Rather than a cut back to a bud, the shoot, stem or branch is cut back to a lateral branch.

objective is to increase the canopy size the bud should be pointing outwards. If pruning is done to induce new growth to fill in a section of the canopy, choose a bud that is pointed in the appropriate direction. The direct effect of this type of cut is vigorous growth of the buds immediately below the cut.

By removing the terminal portion of a shoot or stem, the terminal bud is removed. This is accomplished in both pinching and heading back. The terminal bud is a source of auxin which suppresses lateral bud growth as it is transported basipetally down the shoot or stem. By removing the source of auxin, apical dominance is reduced and buds 15–20 cm below the pruning cut are invigorated. If the shoot or stem is at a 45–60° angle, buds further down the shoot or stem will be invigorated.

The cut should be made at a slight angle about 0.6 cm above the selected bud. The angled cut allows water to run off the cut, facilitating quicker healing. Make sure the angle is not too great, since this creates a larger wound. Wounds take longer to heal if more than 0.6 cm is left above the bud.

Bench cut

A bench cut is a modified heading back cut (Fig. 13.4). Rather than a cut back to a bud, the shoot, stem or branch is cut back to a lateral branch. Bench cuts do not induce vigorous bud growth. When young trees are pruned using a bench cut, the stem below the cut often stiffens creating a stronger branch. Bench cuts are often used to encourage outward-growing limbs. However, spreading young limbs is a better alternative to a bench cut, since bench cuts often lead to watersprout production. Watersprouts are vigorous, upright-growing shoots that develop in response to pruning or wounding. In addition, the lateral just below the bench cut is often weak and breaks under any stress. Bench cuts should only be used if there is no other solution to a problem.

Thinning-out cut

A thinning-out cut removes an entire shoot, stem or limb at its point of origin (Fig. 13.5). It is not invigorating. Thinning-out cuts are used to reduce interior canopy shading and remove excessive growth without stimulating new shoot production. When large limbs are removed using a thinning cut, the three saw cut approach is used to minimize injury to the tree. In a three saw cut, the first cut is made on the lower side of the limb about half way through the limb several centimeters out from the point of origin. The second cut is made on the top of the limb all the way through the limb, several centimeters out on the limb from where the first cut was made. The limb will usually break off due to its own weight during the second cut. The third cut removes the stub at the point of origin to achieve the desired thinning cut.

Hedging and topping

Hedging and topping are non-selective mechanically made pruning cuts to the tops (topping) or sides (hedging) of a tree or shrub canopy. Both are

Fig. 13.5. A thinning-out cut removes an entire shoot, stem or limb at its point of origin.

used extensively in landscape maintenance, citrus (*Citrus* spp.), avocado (*Persea americana*), and to a lesser degree in other fruit crops such as peach (*Prunus persica*), apple (*Malus domestica*), grape (*Vitis* spp.), and blueberry (*Vaccinium* spp.), as well as others. The main drawbacks to topping and hedging are the indiscriminate cuts that are made during the process and the potential for spread of disease inocula.

Hedging consists of cutting back the sides of canopies to reduce canopy width and enlarge the space between rows. When hedging is used, it should be performed regularly so that only small branches are cut during trimming. Don't wait until crowding becomes a problem in the orchard, hedge to prevent overcrowding. If too much growth is removed, excessive vigor may be induced, exacerbating the crowding problem and reducing yield.

Hedging is most often done on an angle of 10–15° such that the top of the canopy is narrower than the bottom giving it a triangular appearance. Yield in fruit crops is often reduced the year following hedging, but recovers after that. The frequency of hedging depends on the crop, tree vigor, and grower preference, but is normally on a yearly or every-other-year schedule.

Topping consists of removing vegetation from the top of the tree canopy at a predetermined desired height. Yield reduction following topping depends on the distribution of fruiting wood prior to topping. If much of the fruiting wood has migrated to the upper portions of the canopy due to shade-induced reductions in the lower portion, significant yield reductions will occur the year following topping. If most of the fruiting wood is located in the lower canopy, little if any yield reduction will occur following topping. The canopy height should be no more than twice the row middle width to ensure adequate light distribution.

Another major reason for hedging and topping is for canopy size management. Without hedging and topping, canopies may quickly become too large for maintenance operations making orchard or landscape management very difficult.

Wound treatment

Pruning wounds should almost always be left without the application of any wound dressing. Allowing the wound to heal naturally is the best approach. The only benefit to using a wound dressing is to prevent the introduction of pathogens causing Dutch elm disease (*Ophiostoma* spp.) and oak wilt (*Ceratocystis fagacearum*) (Zins and Brown, 2009).

When to prune

Effective pruning begins at planting. When young trees, shrubs, and vines are properly pruned at planting, pruning and training in later years is much easier. Proper pruning at planting leads to a well-structured mature plant. Any broken or injured branches or roots should be pruned at planting. Consult a good reference for specific recommendations regarding pruning at planting for the species being planted. For example, it is important to know whether or not you should cut back the leader at planting, how many buds should be left, how many shoots should be allowed to grow that first growing season, etc.

Pruning is often categorized as either dormant or summer pruning. Most pruning should be done late in the dormant season just before new spring growth begins. Branch selection is easy when there are no leaves to obstruct the view. Dormant pruning is an invigorating process while summer pruning reduces vigor by removing photosynthetic area and energy available for growth. Excessively vigorous growth is removed with thinning-out cuts while other growth is reduced in length via heading back cuts. The severity of pruning varies with each specimen. The goal is to establish a regular modest pruning program to avoid excessive pruning in any 1 year. Excessive dormant pruning leads to overly vegetatively vigorous trees that are not very productive, especially in the case of fruit trees.

Pruning too early in the dormant season may induce growth which is particularly susceptible to freezing injury. All fruit trees should be pruned as late in the dormant season as possible to avoid winter injury induced by pruning. Older trees should be dormant pruned first followed by younger trees as younger trees are more susceptible to winter injury caused by early pruning. Pruning induces flower bud growth (Durner, 1995) and a concomitant loss in bud hardiness. Thus later blooming trees should be pruned first followed by earlier blooming trees.

Some landscape shrubs are grown for flowers that originate in buds formed the previous growing season (e.g. redbud (*Cercis canadensis*), *Forsythia*, honeysuckle (*Lonicera* spp.), azaleas and rhododendrons (*Rhododendron* spp.), and others) thus pruning is usually delayed in these species until after flowering. Other species bloom on the current season's growth (e.g. butterfly bush (*Buddleia* spp.), crape myrtle (*Lagerstroemia indica*), and hills of snow (*Hydrangea arborescens*) to name a few) and should therefore be pruned to encourage vigorous spring shoot growth.

Summer pruning may be performed as soon as buds begin to grow in the spring; however, it is normally done after several inches of new growth have occurred. Summer pruning reduces vigor by removing photosynthetic area and energy available for growth. Summer pruning should only use thinning-out cuts to remove vigorous and upright growth in the canopy. It should be completed early in the summer to avoid a reduction in winter hardiness which can occur with late-season summer pruning. In many cases, summer pruning is limited to removal of tissue damaged by wind or injured by a pest, or to hedging and topping of certain landscape species and specific fruit crops. Local recommendations should be consulted for timing and severity of summer pruning and whether or not it is recommended for the species in question.

Physiology of pruning

With an understanding of the interconnected effects pruning has on plant physiology, horticulturists can make much better decisions regarding when and where pruning cuts should be made. Pruning ornamentals is almost always for aesthetic or plant health concerns. Pruning fruit crops is done to: (i) enhance fruit quality; (ii) promote a balance between vegetative and reproductive (fruiting) growth; and (iii) make crop management easier. Much of the work surrounding physiological responses to pruning has been with fruit crops, thus they will be emphasized here. It doesn't matter whether the species is ornamental or one used in fruit production, the responses to pruning are generally the same.

Pruning: a dwarfing process

Pruning removes wood, buds, and cambial tissue. The wood that is removed represents a store of carbohydrates and nitrogen that could have served as reserves for next year's potential growth. The buds removed during pruning are a loss of potential leaf area and fruit while the cambial tissue removed would have been a source for secondary growth. While shoot growth the year after pruning is often significantly increased in pruned versus non-pruned trees, trunk and root growth are both greatly decreased (Forshey *et al*., 1992). Thus, overall, pruning is a dwarfing process (Faust, 1989).

Shoot growth is stimulated by pruning, particularly with heading cuts, for several physiological reasons. Heading cuts lead to an altered hormone balance among the buds remaining on the shoot after pruning since the terminal bud and one or more subtending lateral buds are removed. Thinning cuts do not lead to alterations since the entire shoot is removed. Pruning with either heading or thinning cuts removes terminal meristems which are a rich source of auxin. Initially following pruning, the overall canopy level of auxin is reduced in pruned versus non-pruned trees. Root tissues continue to produce cytokinins which are transported to the shoots, thus the balance of auxin:cytokinin is shifted in favor of cytokinin, which stimulates shoot growth via increased cell division. As shoots develop, they produce significant amounts of auxin and gibberellins in their rapidly growing apices, especially early in the season, which further enhances shoot growth.

Pruning, particularly with heading back cuts, removes terminal meristems and the associated apical dominance. Thus heading back cuts lead to a proliferation of lateral shoots with a concomitant reduction in shoot growth from elongating terminal meristems. Too many heading back cuts can lead to an extremely bushy canopy which can cause excessive shading.

Heading a shoot near the apex induces five to seven of the uppermost buds below the cut into

growth while heading a shoot close to its base often only invigorates the bud immediately below the cut (Faust, 1989). The response to a pruning cut may be attributed to the growth potential of buds as affected by the position on the shoot. Distal buds on a shoot have a much greater growth potential than basal buds (Faust, 1989) thus when a cut is made above distal buds, they grow significantly because they have more potential to grow than basal buds. Similarly, buds higher in the canopy have a greater growth potential than those lower down. Thus the response to pruning is more prominent for pruned shoots higher in the canopy compared with those closer to the ground. Moving a shoot or branch to a more horizontal position reduces the rate of bud growth regardless of position in the tree or on the shoot (Faust, 1989). By manipulating branch angle and adjusting the intensity or position of cutting on the shoot, the growth of stimulated buds can be controlled.

Pruning reduces yield

When a fruiting plant is pruned, flower buds (potential fruit) are removed which usually leads to reduced yield. Additionally, shoot and fruit growth compete for the products of photosynthesis and the enhanced shoot growth stimulated by pruning reduces the photosynthates available for transport to developing fruit.

Pruning improves fruit quality

Pruning in general increases the relative amount of leaf area per individual fruit thus increasing the supply of photosynthates to each fruit which leads to larger, sweeter fruit. Even though the percentage of blossoms which set fruit may be greater on pruned compared with non-pruned trees, the total number of blossoms per tree is greatly reduced on pruned trees. Thus even though a greater percentage of blossoms on pruned trees set fruit, the total number of fruit is much less than on non-pruned trees. The increased light penetration into the canopy afforded by pruning improves fruit color and thereby improves fruit quality.

Pruning delays fruiting in young trees

Pruning stimulates vegetative growth, particularly in young trees, thereby delaying the onset of flowering and fruiting (Faust, 1989). While this may seem undesirable from an economic standpoint, it is imperative to prune for training regularly and intensively early in the life of an orchard. A strong framework is important for production. Additionally, younger trees do not have the limb strength or the photosynthetic capacity to support a large crop.

Physiological effects of summer pruning

Summer pruning is a very selective process performed on a limited number of horticultural crops. Pruning of annual crops during the growing season is not usually considered summer pruning. Summer pruning is selective removal of actively growing shoots of perennial crops such as peaches (*Prunus persica*) and citrus (*Citrus* spp.). When summer pruning utilizes hedging equipment, it is not very selective, but is still considered summer pruning. Specific responses to summer pruning vary with: (i) species; (ii) time of pruning; (iii) severity of pruning; (iv) tree vigor; and (v) geographical location.

Summer pruning should only be practiced with a thorough understanding of its consequences. Summer pruning reduces photosynthetic leaf area thus reducing the photosynthates available on a whole plant basis for both vegetative and fruit growth (Forshey *et al*., 1992). While it is true that summer pruning often stimulates vegetative regrowth, much of the photosynthate produced in new tissue is used for growth of the new tissue and is not exported for fruit growth. Overall, summer pruning leads to smaller trees compared with non-pruned or dormant-pruned trees.

Summer pruning is often used to improve fruit color by increasing light penetration into the fruiting canopy. Fruit color quality is improved, but taste quality may be compromised by reduced sugar levels in fruit from summer-pruned trees (Marini and Barden, 1987).

Summer pruning is often used to reduce crowding in dense plantings. This should only be done as a temporary solution to excessive vigor, as summer pruning may induce vigorous regrowth thereby exacerbating the problem or it may induce fruit or wood sunburn. Plant density should be managed via other horticultural practices such as rootstock selection, fertility management, etc. Root suckers and watersprouts should be removed as they appear as they serve no purpose in the field and may promote and harbor pest problems such as fireblight (*Erwinia amylovora*) or woolly apple aphid (*Eriosoma lanigerum*).

Pruning and photosynthesis

Dormant pruning reduces the potential leaf surface area of a plant. Thus one might think that pruning reduces the overall photosynthesis of a plant compared with a non-pruned specimen. Initially, the total plant photosynthesis may be lower on pruned versus non-pruned trees, however, new shoot growth that is stimulated by pruning more than makes up for the lost leaf area induced by pruning so that by midsummer, total plant photosynthesis of pruned and non-pruned trees is similar (Forshey *et al*., 1992). Dormant pruning may lead to larger leaves with larger mesophyll cells, and increased chlorophyll content on a leaf area basis when compared with non-pruned trees (Faust, 1989). Stomata may also stay open longer during the day in leaves on dormant-pruned versus non-pruned trees (Aldrich, 1935).

Summer pruning before terminal bud set may exhibit a photosynthetic response similar to that with dormant pruning, since shoot regrowth may occur after pruning. If summer pruning is performed after terminal bud set, overall photosynthesis is reduced in pruned compared with non-pruned trees since the actual leaf area removed is not replaced by regrowth. This is significant because carbohydrate production later in the season is reduced with summer pruning and may have negative impacts on winter hardiness and/or shoot growth the following spring.

Better light penetration into the canopy due to either dormant or summer pruning may lead to increased photosynthesis, particularly in leaves that are more interior in the canopy. This may help compensate for the reduced photosynthesis caused by pruning-induced reduction in leaf area.

Training

Training begins at planting whether the crop is annual or perennial. Training is initiated to ensure a well-structured plant that is capable of maximum productivity of high quality product. This might be apples (*M. domestica*), or it might be the display of flowers in an ornamental setting. Delayed training often results in less-than-desired results, poor structure, and weak specimens.

While training often focuses on perennial species, some annuals and biennials are also trained. This includes annuals such as tomatoes (*S. lycopersicum*) and cucumbers (*C. sativus*) and biennials such as raspberries and blackberries (*Rubus* spp.). The reasons for training are the same for all life cycles.

The main reason for training is to develop a strong plant framework from which a large crop can be efficiently produced with minimal limb breakage and maximum light interception. Limb breakage often reduces the productive life of a planting. High light penetration into the interior of a canopy leads to good flower bud formation and the production of high quality flowers and fruit, depending on the commodity. Open canopies also allow for good air circulation which helps reduce disease problems and allows maximum penetration of any pesticide sprays.

Another reason for training is for aesthetics. This is true for both ornamental and non-ornamental situations. A nice looking production system provides a certain level of satisfaction that is hard to describe. Poorly trained and disheveled plantings are undesirable.

Training systems

Ornamentals

Ornamentals are trained primarily for aesthetics based on the desired final form. Selection of appropriately sized species for the landscape under consideration can minimize the amount of pruning and training required, especially in later years. Generally ornamentals are trained as a bush (multiple co-dominant trunks) or a tree (one central dominant trunk or several co-dominant trunks). Once a final form is selected, limb distribution along the trunk(s) is considered. Windbreaks or screening plantings will maintain many limbs lower on the main trunk(s) while specimen trees will gradually shift the bulk of scaffold limbs higher and higher on the main trunk(s). Removal of limbs on specimen trees should not occur until the limb is 2.5 cm in diameter. Leaving lower limbs on the main trunk(s) until they are this size will result in a larger diameter trunk. If lower limbs are removed too soon, a thinner, weaker main trunk will develop. A mistake many people make with a newly planted ornamental specimen is to remove all the lower branches at planting resulting in a long slender trunk with a bushy apex. Leave the lower branches until they are 2.5 cm in diameter.

Ornamental trees should be trained to one dominant trunk or central leader unless the species is normally grown with several co-dominant trunks.

Selection of the central leader and subsequent scaffolds should ideally begin in the nursery. Branches selected to become the permanent structures on a tree should be at an angle of 60–70° from the trunk. This leads to a strong point of attachment and much less chance of breakage. Branches with smaller angles are much more likely to break. Major scaffold limbs should be spaced at least 20 cm but preferably 60 cm apart vertically along the trunk. Five to seven branches should be spaced radially around the trunk to form the final canopy. This radial and vertical spacing sets the stage for a well-structured mature canopy. Trees should be trained and pruned every year or two until the desired final form is attained. Waiting to prune every 4 or 5 years often leads to excessively vigorous regrowth which must then also be pruned.

Smaller trees and bushes may be trained to their natural form or as topiaries or espalier. If a topiary or espalier is desired, make sure an appropriate species is selected. Smaller leaved species such as boxwood (*Buxus* spp.), yaupon holly (*Ilex vomitoria*), or natal plum (*Carissa macrocarpa*) often produce nice topiaries. *Pyracantha, Fatshedra, Magnolia*, yaupon holly (*I. vomitoria*) and others are well suited to espalier training. Sometimes species that most consider as large shrubs can be trained into small trees if desired, by removing lower branches from the trunk(s) over several years. The longer lower branches remain on the trunk, the sturdier the main trunk will be at maturity. Species well suited to this type of training include *Photinia*, wax myrtle (*Morella cerifera*), and *Pittosporum* (Gilman and Black, 2005).

Fruit trees

Fruit tree training normally considers two major types of training based on selection and placement of scaffold limbs. These two major categories include: (i) central leader training; and (ii) open vase or open center training (Fig. 13.6). While they are two very different approaches to tree training, they are both seeking the same outcome: a well-structured tree capable of consistently supporting and producing a large, high quality crop of fruit.

LEADER SYSTEMS The most popular type of leader training system is called the central leader system. A tree trained via a central leader is characterized by one main, vertical trunk called the leader and several whorls of scaffold limbs starting at about 60–90 cm above the ground that are increasingly shorter as height increases which allows maximum light penetration into the canopy. Each layer of scaffold limbs is 45–60 cm above the one below it. Each scaffold whorl consists of three or four well-spaced branches that are not directly opposite any other scaffold limb on that level and does not align with scaffolds of any other level. This ensures minimal shading and maximum light penetration. The number of scaffold layers is determined by the final desired tree height. A well-developed central leader tree looks very much like a Christmas tree. Crops that are often trained to a central leader include apple (*M. domestica*), cherry (*Prunus* spp.), pear (*Pyrus* spp.), plum (*Prunus* spp.), and pecans (*Carya illinoinensis*).

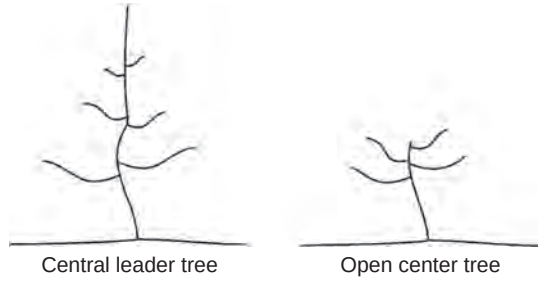


Fig. 13.6. Central leader training versus open center training of fruit trees.

Most fruit trees are purchased as whips, slender, unbranched dormant trees 1–2 cm in diameter, 120–180 cm long. Each tree consists of a rootstock and a scion. Trees are planted either in the fall or late in the dormant season depending on location. Regardless of planting season, the bud union must remain approximately 5 cm above ground at planting. Just before growth begins in the spring, head back the tree (i.e. cut the young tree using a heading back cut) to 75–85 cm above the soil surface. The lowest whorl of scaffolds will develop from buds that grow 10–30 cm below the heading cut, thus the height at which the whip is headed can be adjusted to suit the grower. Thus the lowest level of scaffolds is normally around 45–75 cm above the soil surface.

During the first growing season, vigorous shoots will develop from lateral buds located immediately below the heading cut down the trunk for about 30–45 cm. From these shoots, one vigorous shoot at the trunk apex will be selected to be the leader. All shoots in the first 10 cm of the trunk immediately below the selected leader must be removed.

Four shoots equally spaced around the trunk in the region 10–30 cm below the apex will be selected to form the first and lowest layer of scaffold branches. These four branches should not originate at the same level on the trunk, but rather be at different levels within the mentioned 10–30 cm region below the apex. These shoots are normally spread to form an angle with the trunk of approximately 60°. Spreading is accomplished using toothpicks or spring-type clothes pins positioned at the junction of the shoot and the trunk. Once shoots have elongated sufficiently, clothes pins can be moved to the ends of the shoots to weigh them down and provide the needed spreading to maintain a 60° crotch angle. Any remaining shoots should be removed from the trunk. For the remainder of the first growing season, summer pruning should only consist of removing any new shoots on the main trunk that are not the leader or scaffolds and any directly upward- or downward-oriented growth on the developing scaffold limbs. Failure to effectively train trees during the first growing season will result in extremely difficult, if not impossible, training and pruning during subsequent years.

After the first dormant season, both dormant and summer pruning will be needed. Dormant pruning involves heading the central leader back to 60–75 cm above the highest branch of the layer of scaffolds developed during the first growing season. Any diseased or broken wood should also be removed. Each lateral branch that was selected as a scaffold limb during the first growing season should be headed back by about one-quarter of its length. This will encourage branching of the scaffold branches. Summer pruning that second growing season will consist of selecting a new central leader from a vigorous shoot at the apex along with three or four shoots to become the second scaffold layer starting at about 45–60 cm above the topmost limb of the first scaffold layer. Shoots between the first and second scaffold layers and between the second layer and the central leader should be removed.

Branches of the second level should be spread with toothpicks or clothes pins. Scaffolds in the first level probably still need spreading with larger spreaders made of 2.5 cm square wood with headless nails embedded in each end to act as a spike. Wood pieces are cut to various lengths, say 15, 30, and 45 cm, to provide different-sized spreaders for different-aged scaffolds. Scaffolds are normally spread for about 5 years.

In addition to spreading, any shoots directly competing with the central leader, or with the leader of each scaffold where the heading back cut was made, should be removed. The best way to think of each scaffold limb is that you are developing a horizontal central leader at each scaffold position. Thus the first level of scaffolds consists of four horizontal central leader branches on which side shoots nicely spaced along the length of each branch will be selected. Once the scaffold limbs have reached their desired length, yearly maintenance pruning replaces training pruning.

Dormant pruning in each successive year follows the same general procedure where a new scaffold level is selected each year until the desired tree height is obtained. Mature trees that were properly trained and pruned during their early years will require minimal pruning. Once the desired number of scaffold layers is established, a vigorous central leader is maintained each year via a heading back cut. Scaffold length is maintained as desired with a bench cut to a lateral about the same diameter as the scaffold itself. Summer pruning in subsequent years is aimed at maintaining good tree structure and promoting light penetration through very selective pruning, spreading and removal of all vigorous upright growth. For more details and an in-depth discussion of central leader training see Forshey *et al.* (1992).

Pears (*Pyrus* spp.) are often trained to a multi-leader system since they are extremely susceptible to fireblight (*E. amylovora*), a devastating disease encouraged by cool, wet conditions. A multi-leader tree is one in which several shoots are developed as central leaders so that if one becomes infected with fireblight, it can be removed. Each individual leader is developed as an independent central leader tree with respect to scaffolds and leaders.

Some apple orchards are considered high density orchards with 1000 or more trees/acre. With such close spacing, trees are kept small using size-controlling rootstocks and modified central leader training such as the slender spindle or vertical axe. Both systems are central leader systems with branches along the entire 1.8–3.6 m length of the trunk. Their canopies are generally around 1 m out from the leader.

OPEN CENTER OR VASE TRAINING Peach, nectarine, and plum trees (*Prunus* spp.) are often trained to open center architecture. In open center trees, the central leader is removed early in the tree's life

producing a tree with an open center rather than a central leader. Three to five scaffolds provide a donut-shaped canopy surrounding the open center.

Open center trees are planted as whips or branched trees. Whips are headed back to about 75–85 cm above the soil surface. Remember to keep the bud union about 5 cm above ground when planting. Shoots will develop from buds 15–22 cm below the heading back cut. Three to five shoots uniformly spaced around the main trunk are selected to become scaffolds. Shoots should be at varying heights along the trunk, not at the same height.

Trees planted with branches are treated a bit differently. Any branch below 60–80 cm on the trunk should be removed. Select three or four branches that are well spaced around the trunk to become scaffolds and head the main trunk to just above the topmost scaffold. If fewer than three branches are available for scaffolds, remove all branches and treat the tree as a whip.

During the first growing season scaffolds should be spread to 45–60° to encourage strong branch angles. All upright growth should be removed. New trees should be checked for spreading and upright growth once a month during the first growing season.

Dormant pruning during the first 3 years should encourage lateral growth on scaffolds. Summer pruning can also be utilized to remove unwanted growth and to direct the scaffold growth rather than waiting for dormant pruning. Pruning of mature trees should remove dead or diseased wood while maintaining tree shape and encouraging moderate vegetative growth. Excessive growth will lead to shading while insufficient growth will limit production by limiting flower bud formation on new growth. Fruiting wood should be maintained as close to the trunk as possible to minimize scaffold breakage caused by excessive fruit around the canopy periphery.

Biennials

Biennial species flower and fruit on second year wood. The first year of growth is strictly vegetative. Pruning and training systems for biennial crops such as raspberries and blackberries (*Rubus* spp.) must produce new first year shoots (primocanes) while at the same time managing the second year wood (floricanes) for fruit production. Once fruit is harvested, floricanes die and are removed from the field at the end of the growing season or the beginning of the following one. Most brambles require some form of supporting trellis for production, making training and pruning a bit more difficult.

New cultivars of primocane fruiting brambles make crop management much easier. In the simplest system, all canes are mowed to the ground in the early spring. Fruit is harvested in the late summer or early fall from primocanes that may or may not be supported by a trellis. Canes are mowed to the ground the following spring and the cycle repeated.

Annual vegetables

Two vegetable crops that are most often trained and pruned are tomatoes (*S. lycopersicum*) and cucumbers (*C. sativus*). Both are produced around the world and are most often grown in a greenhouse or a high tunnel (Hochmuth and Hochmuth, 2012a, b).

TOMATOES Greenhouse/high tunnel tomatoes are normally either beefsteak or cluster (on-the-vine) type, however, cherry and grape tomatoes have found a niche market (Fig. 13.7). Most cultivars

Fig. 13.7. Greenhouse tomatoes (*Solanum lycopersicum*) trained to a single leader.

have an indeterminate growth habit and will flower and fruit along the main stem for 10 months or more on a vine that may reach 12 m long. The vine must be regularly pruned and trained to a trellis system. The usual trellis consists of a single overhead wire (0.24 cm diameter) at about 2.5–3 m above the greenhouse floor suspended from two strong endposts. Posts are often located every 9 m or so to help support the fruit and vine load on the wire. Plants are trained up a string that is attached to the overhead wire. Within the row, plant spacing is governed by cultivar vigor, but is generally 45–60 cm. Since vines may grow to 12 m, strings must be long enough to accommodate this growth, with the extra string temporarily attached to the support wire. There are different ways to manage the extra string at the top of the vine at the support wire, but the main objective is to keep it from getting tangled and make it so that it is easy to loosen and tighten as the vine requires.

Each string is loosely attached to the base of the tomato plant stem when the plant has six to eight leaves. Plants are attached to the string with clips, vinyl tape looped around the string and stem fastened with a staple, or by gently wrapping the string around the stem being careful not to cause scraping or crushing of the stem. All lateral shoots (suckers) are removed every 3 or 4 days by pinching or snapping them from the leaf axil from which they originate. Vines also require leaf pruning and it is normally done when vines are lowered as the season progresses. Once the vine has reached the trellis wire, it must be lowered, string and all, so that the indeterminate vine can continue to grow and flower. Generally before lowering, four to six of the oldest leaves are carefully removed from the base of the vine and any old clusters from which fruit has been harvested removed. Plants are then lowered about 45–60 cm and the vine gently coiled or serpentined on the greenhouse floor. There should be 20–25 cm of air space between the greenhouse floor and the lowest set of leaves. The string is then reattached to the support wire. Leaf pruning and vine lowering is performed once every 2 weeks.

Most tomatoes set from one to ten flowers per cluster and should be thinned after fruit set to three to five fruit per cluster, depending on cultivar. Some cultivars may not require thinning.

CUCUMBERS Greenhouse cucumbers are normally standard European seedless, parthenocarpic, gynoecious cultivars producing fruit that is around 35 cm long, slender, seedless and thin skinned (Hochmuth and Hochmuth, 2012b).

Greenhouse cucumber seed is usually expensive at $0.75 each. Germination is high and cultivars are productive. Seedlings with three or four leaves are transplanted and spaced according to training system adopted. Generally a cucumber plant requires 0.46–0.65 m^2 of space to be productive.

A widely used trellising system used for greenhouse cucumbers is a vertical cordon that converts into an umbrella (Fig. 13.8). The main stem of the plant is trained vertically up to an overhead wire positioned about 3 m above the greenhouse floor. Plants are arranged in either: (i) single rows spaced 1.5 m apart; or (ii) double rows (with a space of 0.6 m between the two rows of a pair) and a distance of 1.8 m from the center of one pair of rows to the center of the next pair of rows. Plants are spaced about 45 cm apart within the rows. Double rows will require two support wires.

Plants are trained up strings attached to the support wire and all lateral branches are removed until

Fig. 13.8. Greenhouse cucumbers (*Cucumis sativus*) in the initial stages of training to a vertical cordon system.

the plant reaches the support wire. When one or two leaves have developed on the main stem above the support wire, the growing point of the main stem is removed. Two laterals are allowed to develop and drape over the support wire and grow downwards creating the umbrella modification. The growing point of each lateral is removed when the lateral reaches the greenhouse floor. Fruits develop at the node of each leaf. Fruit developing on the basal 75 cm of the vine are removed. This allows the vine to develop the appropriate vegetative growth to support a full crop. Productivity of the draped laterals is usually less than that of the main stem. Cucumbers are usually harvested about 14 days after fruit set.

Another popular trellising system is the V cordon. Single rows of plants are spaced 1.5 m apart with plants spaced 30 cm apart within the row. Two overhead support wires are fastened above the row, with each wire 38 cm away from the row center. Vines are then alternately trained to the two wires, growing away from the row center. Fruit then hangs nicely making harvesting easier and preventing fruit from rubbing on the main stem or lateral. Vines are pruned similarly to the vertical cordon/umbrella system.

14 Grafting and Rootstocks

Individual plants of many horticultural crops are multiple genetic specimens. Rather than consisting of one single genotype, they are two or more distinct, different genotypes joined together as a single plant. The multiple components are identified as: (i) the rootstock; (ii) the interstem; and (iii) the scion. Grafting (which includes budding) is the process of combining these components in such a way as to establish vascular continuity between them to produce a composite genetic organism that grows as a single plant. Grafting may be natural or actively accomplished by humans. Most of this chapter will deal with forced grafting with only a brief discussion of natural grafting.

The rootstock, stock, or understock is that component of the plant that fuses with the scion and provides the plant's root system. Stock is synonymous with both rootstock and understock, however, understock implies that the lower portion of the plant provides both the root system and some of the trunk. Rootstock implies that only the root system is provided by the lower piece. Rootstocks are chosen for many reasons including soil adaptability, dwarfing, pest resistance, precocity and many others. In some cases when grafting is performed high on the trunk of the rootstock, the rootstock may also provide the trunk and even scaffold limbs in some cases to the finished plant.

The scion produces the plant's shoot system and is the component that produces the desired commodity in most cases, which are usually flowers or fruit. In perennials, the scion is almost always vegetatively propagated. In grafted vegetables, the scion is usually propagated via seed.

An interstem is the third genetic component of some grafted plants and is often selected to provide compatibility between the rootstock and the scion. It may also impart desired characteristics to the scion or rootstock.

Grafting is the general term given to the process of combining dissimilar genotypes into one plant. Budding is a form of grafting in which a single vegetative bud is used as the scion or interstem. Grafting often implies that one or more buds on a common stem piece are combined with the rootstock or interstem.

Perennial ornamental and fruit crops are the standard grafted crops that are familiar to most horticulturists. Several annual vegetable crops are increasingly being grown as grafted plants and interest in using them in commercial production is increasing rapidly.

A good rootstock should possess as many of the following characteristics as possible that are appropriate for the crop: (i) is affordable; (ii) has long-term graft compatibility; (iii) is easily propagated; (iv) promotes precocity and productivity; (v) controls scion vigor; (vi) conveys pest resistance; (vii) improves stress tolerance; and (viii) has minimal suckering.

It is far beyond the scope of this text to cover all crops that may be grafted and their rootstocks. A general discussion of grafting and rootstocks follows with specific references to commonly grafted species.

Reasons for Grafting

There are many reasons for grafting (Fig. 14.1). The main reason for grafting is the clonal propagation of cultivars for commercial production. The story behind the development of apple rootstocks is a good illustration of why grafting developed and how horticultural technology often initially addresses one problem then expands in many directions to enhance production in many ways.

Apple (*Malus* spp.) is difficult to propagate via cuttings and does not come true from seed. To facilitate the propagation of scions desired for their fruit quality and productivity, grafting (and budding) were developed. In propagating desired scions by grafting, fruit growers soon realized that grafted trees came into production much sooner

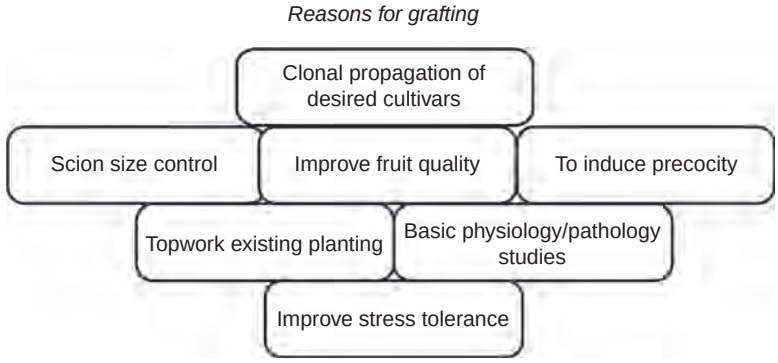


Fig. 14.1. Reasons for grafting.

than seedling trees. Seedlings often took 15–20 years to begin producing a full crop, grafted trees reached full productivity in as few as 5 years. In addition, some grafted trees tended to be smaller than seedling trees. Smaller trees are easier to manage and more of them can fit onto a unit of land. Land use efficiency was increased in both space and time. From the need to efficiently propagate desired scions, and entire industry was changed.

The 'original' apple rootstock, 'Paradise', was selected in the 16th century. The 'French Paradise' rootstock induced precocity and caused scion dwarfing. The 'English Paradise' or 'Doucin' rootstock was less dwarfing. Over time, these rootstocks were mixed, mislabeled and became part of a mixture of genotypes collectively called 'Paradise'. By the 1800s, 14 different strains of 'Paradise' rootstocks existed (Rivers, 1865).

To clear up the confusion surrounding the different strains of 'Paradise', an English researcher at the East Malling fruit research center in Kent, England reclassified samples (29 from Britain, three from France, one from Germany and one from The Netherlands) using botanical and anatomical comparisons. Each stock was evaluated for size control, propagation, and productivity. Eventually 27 rootstocks were released with the East Malling designation, originally EM.1–27 which was changed to M.1–27. Rootstocks were also classified according to their effect on scion vigor and were renamed to remove the confusion surrounding the clones. Rootstocks were classified as: (i) dwarfing; (ii) semi-dwarfing; (iii) vigorous; and (iv) very vigorous (Hatton, 1917). Crosses were made between M rootstocks and the 'Northern Spy' apple at the John Innes Institute in Merton England to introduce resistance to the woolly apple aphid (*Eriosoma lanigerium*) to produce the M.M. series of apple rootstocks, Malling-Merton 101 through to 115 (Jackson, 2003).

Thus grafting originated to obviate problems surrounding the inability to propagate desired varieties of plants directly from seed or obtain clonal plants via cuttings. Over time many other benefits of grafted plants were observed and are now noted as reasons for grafting.

Low success rates or prohibitive costs associated with cuttings, tissue culture or seed propagation may warrant grafting. Grafting to avoid an unfruitful juvenile period is a major catalyst for grafting fruit and nut species. Topworking, a specific form of grafting, is used to change the scion cultivar of an established planting when market demands or production trends suggest a change would be prudent. Pollinizers can also be grafted into established trees to improve fruit set. Injuries can often be repaired by grafting. Disease indexing of stock plants and studies of basic plant physiology, especially long-distance signal transmission in plants, both utilize grafting.

Production Traits Affected by Grafting

There are a number of production traits that may be affected by grafting. These include: (i) improved biotic and abiotic stress resistance; (ii) size control; (iii) induction of precocity; (iv) influence on time of bloom; (v) accelerating plant growth rate thereby reducing nursery production time; (vi) obtaining special plant forms such as weeping cherries or tree roses; and (vii) improved fruit and nut quality.

Size control

Many rootstocks influence scion growth and ultimate canopy size. Larger trees tend to have a larger root system and the dwarfing effect of different rootstocks may (Fernandez *et al*., 1995) or may not (Atkinson *et al*., 1999) be related to the size of their root system. The mechanism for dwarfing is probably very complex and no single unified theory for how it is accomplished has yet been developed.

Many studies on the vascular systems of grafted plants have been performed in the hope of elucidating some of the mechanisms for rootstock action, particularly on size control of the scion (Beakbane and Thompson, 1947; Simons, 1986; Misirli *et al*., 1996). There is good evidence that there are measureable differences in the vascular anatomy of different rootstocks, particularly in the xylem, which may reduce water movement to the scion and thereby dwarf it (Beakbane and Thompson, 1947; Simons, 1986). In addition, root systems that dwarf the scion have a lower xylem to phloem ratio than root systems that do not dwarf the scion (Beakbane and Thompson, 1947). Rootstocks which are not dwarfing also have larger vessel elements than those that are dwarfing.

Dwarfing rootstocks reduce the hydraulic conductivity of the stem xylem (Atkinson *et al*., 2003) and dwarfing rootstocks have increased resistance to water movement at the graft union itself than non-dwarfing rootstocks. These differences in conductivity due to differences in vascular anatomy may be related to limited auxin transport across the graft union (Soumelidou *et al*., 1994). Auxin plays a vital role in the redifferentiation of xylem from callus that forms at the graft union (Hess and Sachs, 1972; Parkinson and Yeoman, 1982; Weatherhead and Barnett, 1986; Savidge, 1988; Uggla *et al*., 1996) and any aberration in auxin levels would certainly alter the redifferentiation process. Signals from the scion, particularly auxin, move more slowly in dwarfing rootstocks compared with non-dwarfing rootstocks (Kamboj *et al*., 1997) even though auxin moves mostly in the phloem, and there is more phloem in scions on dwarfing stocks compared with non-dwarfing stocks (Beakbane and Thompson, 1947).

In many scion–rootstock combinations, scion growth is reduced relative to non-grafted plants and the efficiency in which dry matter can be partitioned into fruit is increased with more dry matter going into the fruit and less going into vegetative growth. This attribute is called yield efficiency and is measured as the weight of fruit produced per unit of trunk cross-sectional area measured at 30 cm above the orchard floor. Different scions on the same rootstock often have different yield efficiencies.

Two key attributes of any rootstock are:

1. What is the degree of dwarfing compared with a standard seedling tree?
2. What is the increase in yield efficiency?

Both attributes combine to establish the appropriate planting density for each scion–rootstock combination. If plants are spaced too closely yield may decline due to interplant competition. If plants are too far apart, they won't fill their allotted space and productivity per unit land area suffers.

Reduced scion growth makes plant management easier. All production operations (pruning, thinning, harvesting) can be performed from the ground without introducing ladders into the field. It takes time to walk up and down ladders and to move them from tree to tree. Ladders also increase the chances for accidents in the field. Smaller trees also require less pruning. Smaller, more compact canopies make foliar sprays of pesticides, growth regulators or fertilizers easier to apply and require less material per application.

Induction of precocity

Most scion budding or grafting stock is mature tissue and has outgrown the juvenile stage in which flowering is lacking. Even so, many rootstocks are known to induce flowering and fruiting in many scion cultivars whether the tissue is juvenile or not. Early and consistent flowering is desirable to bring a commercial operation into production as early as possible thereby making it more sustainable.

Fruit quality control

Fruit quality is a matter of consumer preference. Specific measurements can be made on chemical composition and physical characteristics to quantify quality attributes. The quality of deciduous fruit crops is affected by the rootstock due to rootstock effects on growth characteristics directly related to crop load and canopy management (fruit size, firmness, skin color) rather than direct effects of the rootstock on specific chemical quality parameters (Castle, 1995). On the other hand, in semi-tropical and tropical crops such as citrus (*Citrus* spp.) and

avocado (*Persea americana*), the rootstock can affect fruit quality by directly affecting chemical components of quality. These components of quality are largely a function of water relations within the plant that are affected by the rootstock.

Topworking

Grafting is often used to change the cultivar in an established planting without having to replant. Topworking consists of removing the established cultivar through severe pruning and establishing the new cultivar via grafts onto the stubs left by pruning. Another variation to topworking is the incorporation of a pollinizing cultivar intermittently throughout an orchard, rather than planting entire trees of the pollinizer.

Stress tolerance

The rootstock can influence a plant's tolerance to many forms of stress encountered during production. The tolerance may be a direct tolerance of the rootstock to the stress or an alteration of a scion characteristic by the rootstock that makes the scion more tolerant of the stress.

A rootstock's tolerance to the soil in which it grows is important. Rootstocks vary in their ability to anchor the plant in the soil and thus many crops require support in the form of a trellis or individual stakes. Tree anchorage is often related to brittleness of roots and depth of rooting. For example, trees on the apple rootstock M.9 are very poorly anchored due to brittle roots (Webster, 2002) probably caused by short fibers and a high proportion of root bark to xylem (Ferree and Carlson, 1987). To improve anchorage, scions are often budded higher on the rootstock so that the tree can be planted deeper, allowing root development along the rootstock shank. Rootstocks also differ in their tolerance of chemical properties of the soil such as pH, salinity, sodicity, or nutrient excesses or deficiencies.

Physical soil properties such as texture and water status influence productivity. Some rootstocks tolerate fine or coarse soils and wet or dry soils better than other rootstocks. Rootstocks are often utilized for their ability to impart tolerance of drought and flooding conditions. As water rights becomes a greater issue in agricultural production and climate change impacts water availability, the importance of rootstock choice may become even greater. While dry soil conditions are often overcome with irrigation, flooded soil conditions are often much harder to rectify. Rootstock influence on a plant's ability to tolerate waterlogged soil is especially important in crops that are intolerant of such conditions (*Prunus*) and less important for tolerant crops (*Vitis*). The mechanism by which certain rootstocks impart tolerance to flooded soil conditions seems to be associated with their ability to form adventitious roots (Anderson *et al*., 1984). Adventitious roots have extensive aerenchyma tissue which would allow oxygen to enter the rhizosphere during flooding (Al-Husainy and Jackson, 2001).

There are many soil-borne pests that negatively impact plant growth. One of the most widely observed effects of rootstocks is their resistance to specific pests. Many of these diseases have particular economic importance to the fruit industry. They include: (i) bacteria, e.g. fire blight (*Erwinia amylovora*); (ii) fungi, e.g. crown rot (*Phytophthora cactorum*); (iii) insects, e.g. woolly apple aphid (*Erisoma lanigerum* Hausm.) and grape phylloxera (*Daktulosphaira vitifoliae*); (iv) viruses (citrus trisetza virus in citrus); (v) and nematodes (*Meloidogyne* spp. on peaches and walnuts). The resistance of the rootstock to the pest imparts increased productivity to the scion by virtue of the fact that the rootstock functions more efficiently. In addition, peach rootstocks are chosen for their resistance to the orchard replant syndrome, a malady that prevents the successful re-establishment of peaches on land previously planted with peaches. The mechanism for this resistance and resistances imparted to the scion are poorly understood.

Winter injury to both rootstock and scion are important in colder regions. Rootstocks vary in their hardiness from not hardy to extremely hardy, usually depending on their region of development and adaptation (Quamme, 1990; Quamme and Brownlee, 1997). The rootstock can also influence the hardiness of the grafted scion (Rollins *et al*., 1962; Durner and Rooney, 1988; Durner, 1990a). The mechanisms for such differences in hardiness may be related to the rate of scion maturity in the fall or regrowth in the spring (Ferree and Carlson, 1987). Early-blooming scion cultivars are undesirable in regions where late spring frosts regularly occur. A late frost can totally eliminate a crop. Rootstocks can influence the time of scion bloom in many fruit crops (Durner, 1990a). Even several days delay in bloom to avoid frost or freeze injury can mean the difference between little or no damage and complete crop failure.

Plant physiology/virology investigations

Many horticulturally useful plants are hosts to viruses that may significantly decrease the vigor or productivity of the plant, but otherwise may be present in the plant asymptomatically. If a sample of the suspect plant is grafted onto a sensitive relative that is susceptible and readily shows symptoms of the virus, the virus will be transmitted across the graft union and the presence of the virus can be verified. This procedure is called virus indexing. It is used less frequently now with the availability of specific enzyme-linked immunosorbant assays (ELISA) or assays based on the polymerase chain reaction (PCR).

Poinsettias (*Euphorbia*) are induced to branch by grafting a scion onto the desired cultivar to transmit a phytoplasma (cell-wall-less bacterium) which induces the desired branching (Mudge *et al*., 2009).

In apples, virus infection of rootstocks may reduce scion vigor (which is often desired) as compared with scions grafted onto virus-free rootstocks. For example, scions on virus-free EMLA.9 rootstock are more vigorous than those on virus infected M.9 clones (Autio *et al*., 2001).

Grafting has been used extensively in basic plant physiology studies, particularly to study the translocation of signaling molecules and nutrients within the plant. Some examples include: (i) the translocation of secondary metabolites (Wilson, 1952); (ii) the flowering stimulus (Zeevaart, 2006); (iii) the potato tuberization stimulus (Ewing and Struik, 1992); (iv) plant hormones (Foo *et al*., 2007); and (v) RNAs (Tournier *et al*., 2006).

Rootstock Propagation

The ease of propagation was once the criteria for selection of many rootstocks. As more reliable means of propagation were introduced (Fig. 14.2), criteria for selection became more diverse to include vigor control, pest resistance, adaptability to soils and climate, etc.

For much of history, rootstocks were propagated from seeds collected from wild indigenous populations and scion performance on these seedlings was extremely variable. In the 17th century, clonal propagation of selected seedlings began (Webster, 1995). The first propagules were probably suckers arising from the base of superior trees. Eventually, methods of layering and stooling became popular and only rootstocks that could be easily propagated with these methods were selected. As selection criteria shifted from ease of propagation to greater adaptability and sustainability, rootstocks have been selected that are often not easy to propagate.

Seeds

Most rootstocks grown throughout the world are propagated by seed (Webster, 1995). Many fruit crop rootstocks including peaches, nectarines (*Prunus persica* L.), apricots (*Prunus armeniaca* L.), Asian pears (*Pyrus pyrifolia* (Nakai)), and *Citrus* species are propagated by seed. While seed propagation is easier and more economical for the nurseryman, seed-propagated rootstocks are usually far less desirable for the grower since seedling rootstocks are often inferior to clonally propagated

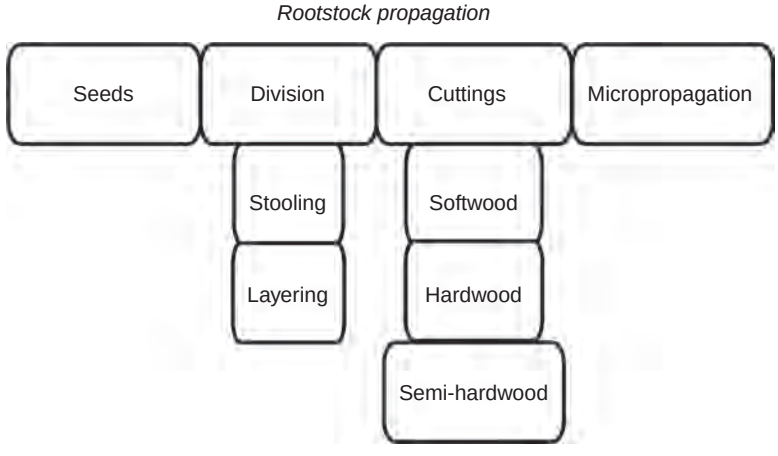


Fig. 14.2. Common methods of rootstock propagation.

ones. However, in species where viruses are not transmitted through seeds, and virus-infected clonal material is evident, seedlings are probably more beneficial. Seedlings also help avoid transmission of root-borne diseases during clonal propagation.

The largest drawback of using seedlings as rootstocks (besides the obvious lack of desirable traits from specific clonal rootstocks) is the lack of uniformity among seedling rootstocks. Uniformity among seedling rootstocks can be improved by using seed of a specific clone or from a self-fertile cultivar well isolated from other cultivars or by using seeds from apomictic rootstocks. Apomictic seeds are widely used in the citrus industry. Apomictic seeds are formed when a cell in the embryo sac fails to undergo meiosis (reduction division) and forms an embryo genetically identical to the seed-bearing plant. Thus apomictic seeds are an asexually derived, clonal seed propagule.

Division

Propagation by division has an advantage over cuttings in that propagules are not removed from the parent plant until after roots are well established on the propagule. The main forms of division used in rootstock propagation are: (i) stooling; and (ii) layering.

Stooling

Rootstock plants that are at least 1 year old in the rootstock nursery are cut, removing most of the above-ground portion of the plant. Shoots arise from buds on the remaining stump. Soil or sawdust is used to mound the bases of these shoots to blanch them and induce rooting. This mounding process is performed several times during the summer until 15 cm of the shoot is covered. Temperature, moisture and adequate oxygen must be maintained around the rootstock stem to ensure success. Pest control is also important. After leaf fall, the mounding material is removed and the rooted stems are removed from the stump to be placed in the budding nursery where the appropriate scion cultivar will be grafted to it. The stump left behind is used again the following season for stooling.

Layering

Layering is similar to stooling except that not all rooted shoots are removed from the parent plant at harvest. Several shoots are left on the parent plant and in the spring, they are bent to horizontal before bud break and held to the soil with pins, then covered with a shallow 3–4 cm layer of soil. Axillary and adventitious buds will grow and their bases will be etiolated since they are soil covered. Rooting will occur in this etiolated zone. Mounding and harvesting are performed as in stooling.

Cuttings

The three main types of cuttings used in rootstock propagation are: (i) softwood (summer) cuttings; (ii) hardwood (winter) cuttings; and (iii) micropropagation. Micropropagation is used mainly for hard-to-root species or for rapid multiplication of rootstocks that are in short supply. Two other forms of cutting occasionally used in rootstock propagation are semi-hardwood cuttings and root cuttings.

In any form of cutting, the parent from which the cutting is taken must be healthy and at the right stage of development. Once the cutting is taken, it may require further wounding or treatment with auxin to stimulate root initiation. A major factor that makes one rootstock easy to propagate and another one extremely difficult to propagate is related to the amount of auxin in the cutting shoot base (Alvarez *et al*., 1989). Difficult-to-root species often have high levels of bound, inactivated indole acetic acid (IAA) in them compared with easier-to-root species. Free auxin, which is at a maximum 1 day after cutting, leads to dedifferentiation of parenchyma cells near vascular bundles in the base of cuttings. These dedifferentiated cells then form root primordia about 100 h after cutting. The final consideration for successful rooting is the placement of cuttings in an environment to encourage rooting.

Softwood cuttings

Softwood cuttings are taken from parent plants early in the growing season when tissues are soft and succulent or just beginning to lignify. They are generally 5–20 cm in length and are removed from the parent just below a node. Leaves are removed from the bottom third of the cutting and the remaining leaves may be trimmed to half their size. Cutting bases are often treated with auxin to stimulate rooting and a fungicide to prevent rot if it is known to be a problem. Cuttings are placed in a moist but well-drained rooting medium usually in a partially shaded greenhouse or lath house with

high humidity maintained with intermittent misting or fogging. Misting or fogging is decreased as rooting progresses and plants are often ready to be transplanted in 4–6 weeks.

Hardwood cuttings

Hardwood cuttings are taken from parent plants either after leaf fall or before bud break during the dormant season. Cuttings taken during the middle of the dormant season usually do not root well. Cuttings taken from severely pruned plants are preferred to cuttings from any other source. Cutting bases are treated with auxin either as a long soak in a dilute (250–500 ppm) solution of auxin or a quick dip in a concentrated (2500–5000 ppm) solution. Auxin in talcum powder may also be used. In all cases, it is important for the cut base to be exposed to the auxin so that adequate uptake can occur.

Many hardwood cuttings benefit from wounding prior to auxin treatment. Wounding most often consists of cutting the base of the cutting longitudinally for several centimeters to expose the cambium, which allows more auxin to enter the cutting and stimulate rooting. Wounding increases both the number of cuttings that root and the number of roots per cutting.

After wounding and auxin treatment, cuttings are placed vertically with their bases inserted into 10–20 cm of rooting material such as sterile compost or sand and kept at 21°C or higher for 8–10 weeks. In most cases only the base of the cutting is heated, usually by heating cables in the medium. Keeping the entire cutting at 21°C or greater may improve rooting, but is often more costly. Cambial cells dedifferentiate into callus tissue which then differentiates into roots.

It is imperative to prevent excessive water loss from cuttings during the initial weeks following placement in the rooting medium. Excessive water loss leads to a dramatic decrease in rooting success. Additionally, once callusing and rooting begin, excess water can be equally detrimental. Once rooted, plants are transferred to the grafting nursery in the spring.

Micropropagation

Rootstocks are often propagated by micropropagation, especially hard-to-root species or those in short supply. There are many different recipes for micropropagation media, but they all consist of a liquid or solid carrier such as agar, along with nutrients, minerals, sugars, vitamins, and hormones. Axillary shoot proliferation is the micropropagation method most usually employed for rootstock propagation. The distal 5 mm of an actively growing shoot are excised and cultured on an appropriate medium. The axillary buds contained in this shoot meristem begin to grow into shoots which are separated from the meristem. Each shoot is then ready to be similarly propagated after several weeks' growth. Using this method, large quantities of new plants can be generated without maintaining large quantities of stock plants. This method also avoids mutations into off-types that are often associated with other tissue culture methods that rely on dedifferentiation of tissue into callus followed by differentitation into shoots then roots via manipulation of hormone levels in the medium.

Once enough shoots are produced via axillary shoot proliferation, root formation is induced on the propagules by altering the growth regulators in the growing medium, usually high auxin and relatively low gibberellins and cytokinins. Once rooted, plants are slowly acclimated to 'normal' growing conditions.

Physiology of Grafting

The physiology of grafting has long fascinated horticulturists. Both the physiology of graft wound healing and the physiology of the effects grafting causes have been investigated for years, however, our knowledge of these mechanisms remains fairly incomplete (Atkinson and Else, 2001).

Basic biology of grafting

The biology of grafting starts with the formation of a graft union between the grafted parts. Union formation is particularly interesting since a number of events must occur in sequence for success. A wound is initiated by the grafting cut which leads to cellular dedifferentiation, cell division, followed by the establishment of a functioning vascular system connecting the two grafted pieces.

The first and most important step in establishing a successful graft union is lining up the cambial layers of the two grafted tissues. The cambium is one cell layer thick, thus the grafter must be meticulous in aligning these two layers during graft placement. Since a layer one cell thick is not easy to see, lining up the region where the cambium is

located is often sufficient for success. To ensure that the cambia remain aligned and maintain contact through the healing process, the two grafted pieces are bound together with rubber ties, twine or similar materials that eventually disintegrate.

The cuts made during grafting induce a wound response in both grafted pieces leading to cell dedifferentiation and callus formation. The scion side of the graft union is often more prolific than the rootstock or interstem side in terms of callus production. Callus from both sides of the graft union must commingle to form a successful callus bridge between the two parts.

The next crucial step is the formation of a vascular bridge between the two pieces via differentiation of cells in the callus that commingled during the wounding response. New cambial cells differentiate from parenchymatous callus cells. These new cambial cells give rise to xylem and phloem; xylem generally forms first followed by phloem. Following the formation of a vascular bridge between the grafted entities, a new vascular cambium must develop to produce secondary xylem and phloem (Hartmann *et al*., 2002). Auxin released from vascular strands in the scion and rootstock stimulate the differentiation of vascular tissue in the wound-induced callus (Aloni, 1987; Mattsson *et al*., 2003).

Many factors influence whether or not a graft union 'takes'. Genotype has a large influence on grafting success, some species are easier to graft than others. In addition, the genotypes of the scion/interstem/rootstock are important in that the closer they match, the more likely there will be a successful union.

Temperature can effect cambial growth and callus formation. In hardwood grafts, plants are often held at temperatures between 4.5 and 5.0°C for several months to encourage callus formation and wound healing without stimulating vegetative growth of the scion. Cooler temperatures inhibit callus growth and wound healing and higher temperatures may lead to excessive callus growth and depletion of carbohydrate reserves within the plant. Moderate temperatures are also required in other types of grafting to promote cambial growth and wound healing.

Moisture must be maintained around the graft to prevent desiccation. Grafts are often wrapped with various materials or covered with wax to prevent desiccation. In some types of grafting, the rootstock must be actively growing. T-budding, chip budding and bark grafting all require that the bark is slipping, which is the result of active cambial growth. The craftsmanship of the grafter is extremely important. Some people are meticulous and are very good at aligning cambial tissues to promote the successful graft union while others are not. Cambial alignment is paramount to grafting success.

Graft incompatibility

When a graft union fails to occur or when a graft union fails to hold after apparent success, the graft is said to be incompatible. Sometimes incompatibility is immediate and the union never occurs. In many cases, the graft appears successful but fails several months to many years after 'taking'. Compatibility should be considered on a continuum from totally compatible to totally incompatible. Some unions which appear incompatible may actually be productive if proper horticultural practices such as training and trellising are used.

A major cause of immediate incompatibility is the failure to make vascular connections between the two grafted pieces, often due to misalignment of the cambia. Aligning grafting cambia is as much an art as it is a science. Sometimes the vascular connection does not occur due to adverse environmental conditions during the healing process. Usually insufficient water supply to the scion or unacceptable temperatures prevent the formation of a vascular connection. Disease can also play a role in causing immediate incompatibility, thus it is important to use only clean stock.

Genetic incompatibility occurs when there is some form of cellular miscommunication between the two grafted pieces, preventing the successful establishment of a union. Many theories have been proposed as the mechanism for genetic incompatibility, however, none of them really target the cause or explain why the union failed but rather describe the symptoms and what happens.

Some symptoms of incompatibility besides the obvious lack of a physical union between the two pieces include: (i) yellowing of foliage after leaf expansion in the spring; (ii) declining vegetative growth over time for no other apparent reason such as disease or poor fertility; or (iii) a clean break of the scion from the rootstock at the grafting site long after the graft appeared to be successful.

In general grafting is most successful with dicots and gymnosperms but some limited success has been achieved with monocots. The more closely related the scion and the rootstock are, the more likely there is to be a successful graft union.

Scion–rootstock hormonal interactions

The interactions observed among rootstocks, interstems and scions are often attributed to alterations in hormone levels throughout the plant. In addition, whether or not the graft is compatible is often attributed to hormonal signaling among the grafted segments.

Auxin is produced in shoot apices and cytokinins are produced in the roots. Decreased auxin transport to the roots leads to an increase in the synthesis and export of cytokinin from the roots to the shoots (Bangerth, 1994). An increase in cytokinins in the xylem sap leads to an increase in auxin synthesis and translocation from the shoots which in turn would decrease cytokinin production by the roots. The balance of auxin and cytokinin between shoots and roots leads to the normally observed balance of growth between the root and shoot (Sorce *et al*., 2002). When the normal balance is altered, the growth observed is altered. One theory is that invigorating rootstocks do so by increasing the amount of cytokinins exported from the roots to the shoots resulting in more robust scion growth (Sorce *et al*., 2002). Dwarfing rootstocks may reduce the basipetal transport of auxin to the roots which reduces the amount of root-produced cytokinin and gibberellin translocated to the shoot, resulting in reduced shoot growth (Van Hooijkonk *et al*., 2010). Greater cytokinin concentration in apple xylem is associated with vigor-inducing rootstocks (Kamboj *et al*., 1999).

Gene transcripts associated with auxin signaling have been shown to move from pumpkin (*Cucurbita maxima* Duch.) rootstocks to melon (*Cucumis melo* L.) scions (Omid *et al*., 2007) providing evidence for signal movement from the rootstock to the scion. Squash (*Cucurbita* spp.) xylem sap contains significant amounts of cytokinin that can inhibit auxin-induced cucumber hypocotyl elongation, suggesting that cytokinins translocated from the roots may alter scion growth by affecting the response to auxin (Kuroha *et al*., 2005).

Incompatibility in cucurbit grafting has been shown to occur at about 25 days after grafting and is attributed to a hormonal imbalance, particularly of auxin and ethylene in the rootstock (Aloni *et al*., 2008). The source of the incompatibility seems to be extreme sensitivity to auxin in the rootstock. Auxin produced in the scion is translocated to the rootstock once the graft connection is made and when it reaches some threshold level in the roots, it triggers inhibited root growth and enhanced root decay. Incompatible rootstocks are either more sensitive to auxin or accumulate more auxin than compatible ones. The degradation induced by auxin may be due to damage from oxidative stress caused by ROS build up, either due to increased production or reduced removal via peroxidases and other enzymes, in incompatible root systems.

High root auxin levels may trigger ethylene production in incompatible rootstocks leading to their degradation (Mulkey *et al*., 1982; Rahman *et al*., 2001). Roots produce increased amounts of ethylene following auxin treatment and incompatible rootstocks produce more ethylene than compatible ones (Aloni *et al*., 2008).

There are other observations illustrating the involvement of hormone signaling in rootstock–scion interactions. Cotton (*Gossypium* spp.) cultivars vary in their time of leaf senescence, some senesce early while others senesce late. When early senescing scions are grafted onto late senescing rootstocks, scion leaf senescence is significantly delayed. When late senescing scions are grafted to early senescing rootstocks, scion leaf senescence is accelerated. Late senescing rootstocks produce cytokinins which are translocated to the scion to delay leaf senescence (Dong *et al*., 2008).

Salt tolerance in potato (*Solanum tuberosum*) and tomato (*Solanum lyycopersicum*) can be induced by grafting salt-intolerant scions onto salt-resistant rootstocks (Chen *et al*., 2003; Dodd, 2007; Etehadnia *et al*., 2008; Albacete *et al*., 2009). The salt tolerance is conferred by enhanced ABA production and subsequent translocation to the scion by the salt-tolerant rootstock.

Watermelon (*Citrullus lanatus*) grafted to squash (*Cucurbita* spp.) roots are more vigorous than non-grafted plants due to enhanced cytokinin production and translocation from the rootstock to the scion (Yamasaki *et al*., 1994). Sweet cherry (*Prunus avium*) productivity on different rootstocks was shown to be positively related to higher levels of cytokinins in scion xylem extracts (Stevens and Westwood, 1984). Higher levels of cytokinin enhance tissue sink strength (Mothes and Engelbrecht, 1961) which may help explain the effect of rootstock on scion vigor and productivity.

Grafting Ornamentals

One of the major problems in identifying problems in ornamental plants is that many species are grafted

and the rootstock is often unknown and is often not considered when trying to diagnose problems (Ball, 2004). It is important to keep good records of ornamental specimens so that in later years, problem diagnosis may be simplified by knowing both the scion and the rootstock. Incompatibility is a serious problem with oak (*Quercus* spp.), maples (*Acer* spp.), yews (*Taxus* spp.), and Douglas fir (*Pseudotsuga menziesii*) in that it is often manifest 4 or more years after planting. This 'delayed' incompatibility has essentially wasted years in developing the desired landscape. A solution is to utilize specimens that are on their own roots.

Even in compatible combinations problems between the scion and the rootstock can occur. Often the rootstock will send up a vigorous sucker that if not removed promptly will outcompete the scion and result in an undesirable plant. For example, crab apples (*Malus* spp.) are often grafted onto apple (*Malus domestica*) seedlings with such results. Root suckers must be removed promptly.

In a number of ornamental species, the rootstock does not end 5–10 cm above the ground, but rather may extend as the trunk of the tree 1–1.5 m. Weeping forms of trees such as tabletop elm (*Ulmus glabra* 'Pendula') and weeping mulberry (*Morus alba* 'Pendula'), are budded to rootstocks about 1.5–2 m above ground (Ball, 2004). The trunks of these trees must be kept free of shoots.

Vegetable Grafting

Vegetable grafting has been practiced for many years in Eastern Asia to alleviate soil-borne pest problems and to enhance plant vigor and productivity under adverse environmental conditions. Grafting vegetables has become a very popular pest management technique worldwide and continues to grow in popularity.

In Japan and Korea, the majority of muskmelons (*Cucumis melo*), watermelons (*C. lanatus*), cucumbers (*Cucumis sativus*), tomatoes (*S. lycopersicum*), and eggplants (*Solanum melongena*) in both field and greenhouse production utilize grafted transplants (Univeristy of Arizona, 2012). Nearly all of the watermelon production in the Almeria region of Spain utilizes grafted plants to control Fusarium wilt. Most grafted vegetables in North America are tomatoes used for greenhouse production; however, grafting heirloom cultivars onto suitable rootstocks for organic production is becoming increasingly popular. As the use of the fumigant methyl bromide is phased out, interest in grafted vegetable transplants is likely to increase.

The vegetable crops most frequently grafted are tomatoes (*S. lycopersicum*), cucumbers (*C. sativus*), muskmelons (*C. melo*), watermelons (*C. lanatus*), eggplant (*S. melongena*), and peppers (*Capsicum annuum*). Before embarking on a fully fledged switch to grafted plants, growers are encouraged to experiment on a small scale and consult with local experts. The major benefits of grafted vegetable transplants include: (i) resistance to soil-borne pests; (ii) tolerance to abiotic stresses such as chilling, heat, and salt stress; and (iii) increased plant vigor and yield. While robotic grafting machines are available, grafting by hand is practiced worldwide.

Tomato grafting

Tomato rootstocks are selected for resistance or tolerance to Fusarium wilt, bacterial wilt (*Ralstonia solanacearum*), Verticillium wilt, tomato corky root (*Pyrenochaeta lycopersici*) and root knot nematodes (*Meloidogyne* spp.). These rootstocks are often hybrids of cultivated tomato cultivars (*S. lycopersicum*) to create intraspecific hybrids or between cultivated tomatoes and wild relatives to create interspecific hybrids. Interspecific hybrids may suffer from lack of germination uniformity, but are often more robust compared with intraspecific hybrids.

Rootstock and scion vigor should also be considered in their selection. A less vigorous scion is expected to become more vigorous if grafted to a vigorous rootstock. The rootstock should not be so vigorous as to cause excessive vegetative growth of the scion which might result in reduced yields.

When dealing with cultivars and rootstocks with resistance to tomato mosaic virus (ToMV), the level of resistance of both the scion and the rootstock must be considered. The scion must be as resistant as the rootstock, or more resistant than the rootstock to prevent a hypersensitive reaction from occurring at the graft union should the scion become infected with the virus. A hypersensitive reaction induces cell death which would lead to a sudden collapse of the graft union, sudden plant death, and total crop failure. Many seed companies classify resistance as tm-1 or tm-2, with tm-2 being more resistant.

Tomatoes are most commonly grafted using the 'Japanese Grafting Method' also called tube grafting (Fig. 14.3). In this method, scion and rootstock

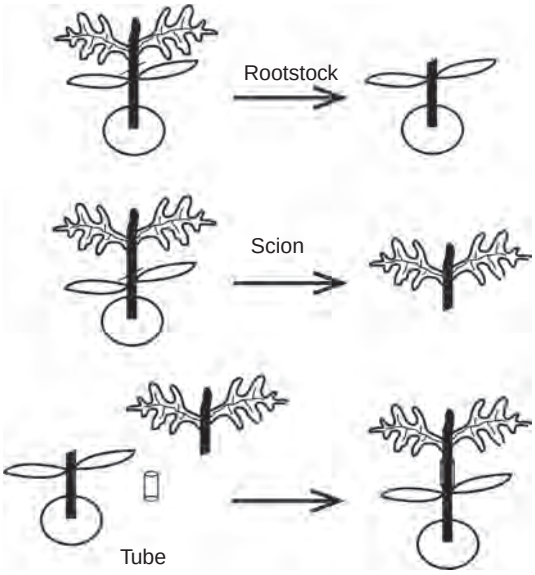


Fig. 14.3. Tube grafting of tomato (*Solanum lycopersicum*).

should both be at the two true leaf stage and their diameters must match (1.5 mm). The rootstock is cut using a razor blade at a 45° angle just above or below the cotyledons. Cutting below the cotyledons is preferred to prevent possible growth of axillary buds. A grafting tube (commercially available, made of silicone) is placed so that half of the tube is on the rootstock hypocotyl (stub remaining after cutting). The scion is cut at the same angle about 5–10 mm below the cotyledons, making sure the scion hypocotyl diameter matches the cut rootstock. The scion is placed in the grafting tube already on the rootstock, making sure the scion and rootstock cut surfaces align and fit tightly. The grafted seedling must be misted regularly to prevent desiccation during healing which occurs over a 7 day period.

Cucurbit grafting

Cucurbit (watermelon (*C. lanatus*), cucumber (*C. sativus*), muskmelon (*C. melo*)) rootstocks are selected for resistance or tolerance to Fusarium wilt and melon necrotic spot virus as well as tolerance to cool temperatures. Cucurbits are usually grafted to interspecific squash hybrids (*Cucurbita maxima* × *Cucurbita moschata*), bottle gourd (*Lagenaria siceraria*), figleaf gourd (*Cucurbita ficifolia*), or other melons (*C. melo*). Small trials for the best scion–rootstock combination should be carried out since fruit quality and scion growth can be greatly affected by rootstock.

Watermelons (*C. lanatus*) are often grafted to interspecific squash hybrids to improve tolerance to chilling. Such plants may be too vigorous and may have delayed flowering and reduced sugar content of fruit. Thus they are often grafted to bottle gourd instead, which is less vigorous but still has chilling resistance. Muskmelons are usually grafted to interspecific squash hybrids or other muskmelons. When grafted to other muskmelons, identification of rogue rootstock fruit may be difficult. Cucumbers are normally grafted to interspecific squash hybrids or figleaf gourd. Some rootstocks improve cucumber fruit quality by reducing silicon deposition on the fruit epidermis (the bloom often seen on cucumber, which is not desirable).

Cucurbits are grafted using one of three methods: (i) hole insertion grafting; (ii) one cotyledon grafting; and (iii) approach grafting.

In hole insertion grafting, the scion and rootstock diameters do not have to match but the scion diameter must be the same or smaller than the rootstock. Scions are usually grafted onto rootstock cuttings rather than onto intact plants. For success, the graft union must heal and the rootstock cutting must form roots. The scion should be at the beginning of the first true leaf stage, around 2–3 mm. Rootstock seedlings should also be at the first true leaf stage (2 cm) with long hypocotyls (7–9 cm). Rootstock cuttings should be harvested about 30 min before grafting to allow them to lose some turgidity. This prevents cracking of the hypocotyl when the hole for insertion of the scion is made. The first true leaf, the apical meristem and any axillary meristems must be removed from the rootstock cutting. A hole is made in the rootstock cutting by inserting a sharpened chopstick or similar device into the axil of one cotyledon and forcing it through the hypocotyl, just below the opposite cotyledon. Cut each side of the scion hypocotyl making a cut edge about 7–10 mm long, but do not create a V at the end of the scion, but rather leave it somewhat wider than a V would be. Remove the tool used to form the insertion hole and insert the scion, cutting into the rootstock cutting making sure the cut surfaces of the scion make good contact with the rootstock tissue. Put the grafted cutting into a propagating tray filled with moistened media. Mist the cuttings to prevent dehydration.

Move the tray to a healing chamber for about 1 week to allow graft healing and rootstock rooting.

A second approach to cucurbit grafting is called the one cotyledon method, originally developed for robotic propagation operations. In this type of propagation, the rootstock may be an intact plant or a cutting. Both the scion and the rootstock should be at the first true leaf stage. Long rootstock hypocotyls (7–9 mm) are preferred if rootstock cuttings rather than intact plants are used. Remove one cotyledon from the rootstock at a 45° angle while at the same time removing the apical and both cotyledonary axillary buds. Prepare a scion with matching hypocotyl width by cutting at a 45° angle about 10–15 mm below the cotyledons. Make the cut perpendicular to the cotyledons so that when the scion and rootstock are grafted, the scion and rootstock cuts are aligned and one of the scion cotyledons is aligned with the rootstock cotyledon. Hold the graft together with an appropriate-sized grafting clip. If utilizing rootstock cuttings, place the newly grafted cutting into new substrate and mist to prevent dehydration. Grafted plants should be misted as well to prevent desiccation. Move the cuttings or plants to the healing chamber for 7–10 days. The grafting clip can be removed once the graft has healed.

Approach grafting can also be used with cucurbits and while slower than other methods, it normally leads to a high success rate even with unfavorable healing conditions. Both the rootstock and the scion should be at the first true leaf stage. Using a sharp razor blade, make a downward cut at a 60° angle from horizontal about half way through the rootstock hypocotyl. Make a similar cut, only upwards, in the scion hypocotyl. Insert the downwards-facing tongue of the scion into the upwards-facing tongue of the rootstock. Hold the graft in place with a grafting clip. Immediately plant both plants in the same container and allow the graft to heal for 1 week. Once the graft has healed, remove the shoot of the rootstock and the roots of the scion.

Eggplant grafting

Grafting eggplants has not been as widely developed as tomato and cucurbit grafting, especially in North America. The rootstocks used for eggplant grafting include eggplant (*S. melongena*), torvum (*Solanum torvum*), and scarlet eggplant (*Solanum aethiopicum*).

Healing grafted seedlings

Graft healing is a critical step in the propagation chain of events and conditions should be maintained as close to those recommended as possible. Specialized chambers or greenhouses are used for healing. The relative humidity (RH) surrounding grafted cuttings or plants should be 95% or higher. As wounds heal, the RH can be allowed to decrease. Grafts should be kept within the temperature range of 28–29°C to stimulate callus formation at the graft union. Grafts should be kept in the dark for 24–48 h after grafting then provided with light either naturally or artificially at a level of 100 μmol/m^2/s PAR. This light level is extremely low, close to the light compensation point which is just enough to maintain the plant during healing. Tomatoes require 4–6 days and cucurbits require 7 days to heal under these conditions.

Mukibat Grafting

One of the most intriguing types of grafting is called Mukibat grafting of cassava (*Manihot esculenta*) which was developed by a peasant subsistence farmer from Indonesia named Mukibat in 1958 (Mudge *et al*., 2009). While most grafting is performed to establish a rootstock effect on a scion, Mukibat grafting is used to establish an invigorating scion effect on the rootstock. In cassava, the roots are harvested for food. Mukibat grafted a relative of cassava, the Ceara rubber tree (*Manihot glaziovii*) onto cassava leading to a 30–100% increase in the yield of cassava compared with non-grafted controls (De Bruijn and Dharmaputra, 1974).

Flower Bud Grafting

Flower bud grafting is a grafting technique adopted by the Taiwan pear industry (Kuniyama, 1996; Gemma, 2002). Asian pears (*P. pyrifolia*) are a favorite fruit in Taiwan, however, some desirable cultivars cannot be grown due to insufficient winter chilling to break dormancy. To overcome this production obstacle, budwood from trees in Japan that have received almost enough chilling to break dormancy is harvested, shipped to Taiwan then held at 2–4°C to complete chilling. The chilled buds are grafted onto 'Heng Shan' pear trees to produce two to four fruit per cluster that demand very high prices. The process is labor intensive and must be performed every spring.

In Vitro Grafting

In vitro grafting is the technique where a 1.5 mm shoot tip from a mature plant is grafted onto a seedling rootstock (Murashige *et al*., 1972). The technique was developed for citrus but is used for many species. Shoot tip grafting is used to clean cultivars of viruses. Plant meristems are free of viruses and other pathogens. When a desired cultivar is infected, clean plants can be produced by grafting meristems from infected plants onto a desired rootstock. The meristem that is grafted is free of the offending pathogen and, since it is from a mature plant, retains its mature status and does not have to go through a period of juvenility before it begins producing fruit. This type of grafting avoids the juvenility present in virus-free seedlings produced from clonally propagated nucellar seeds and cleans stock of pathogens that are resistant to thermotherapy cleaning (Roistacher, 2004).

Natural Grafting

Natural grafting, particularly root grafting, is common. While not used in commercial horticulture, natural grafting has some important ramifications on several economically important horticultural crops.

Two important ornamental diseases, dutch elm disease, caused by the fungus *Ophiostoma ulmi*, and oak wilt disease, caused by *Ceratocystis fagacearum*, are both transmitted via natural root grafts between individuals within each species (Epstein, 1978). Variegated chlorosis (*Xylella fastidiosa*) is a bacterial disease of citrus that can be transmitted between rootstocks of adjacent citrus trees through natural grafts (He *et al*., 2000). Avocado (*Persea americana*) sunblotch disease (Mudge, *et al*., 2009) and xyloporosis of citrus (Epstein, 1978) are diseases caused by a viroid and a virus, respectively, and are transmitted by natural root grafts.

While some consider the 'graft' between mistletoes (parasitic plants of the order *Santalales*) and their host plants natural grafts, they differ from true grafts in one very important aspect. In 'true' grafts, there are apoplastic connections between scion and stock as well as symplastic connections via plasmodesmata (Tidemann, 1989). In the 'graft' between mistletoe and host, no such plasmodesmatal connection has been observed (Coetzee and Fineran, 1989).

Graft Chimeras and Hybrids

One of the interesting aspects of grafting is the possibility of direct genetic transformation of one of the grafted components by another grafted component, known as a graft hybrid. Graft chimeras are often confused as hybrids, but they are not, as seeds produced by such chimeras do not segregate but rather reflect either one or the other of the chimera parents. Graft chimeras occur when the tissue of two distinct genetic components intermingle to present the impression that a new, often abnormal, curiosity has been formed by their union. Examples are often cited such as a lemon grafted to an orange which then produces fruit that are half sweet, half sour or a white rose grafted to a red rose which then produces both red and white flowers.

Graft hybrids would require actual genetic transformation of one tissue, usually scion, by another, usually rootstock. The transformed tissue would then have to produce seed that segregates into the characteristics of both 'parents'. Claims of such grafting-induced genetic transformations have not generally been reproducible. Even though reports have appeared in peer-reviewed scientific journals providing evidence that it does occur, no definitive proof has been offered and the subject remains highly controversial.

15 From Harvest to Market

Employing the finest methodologies in horticultural production are meaningless if commodities are not harvested at the appropriate time and handled in a manner to maximize their longevity during transit and storage. Quantitative and qualitative losses occur from harvest to consumption. Quantitative losses are easy to assess but losses in quality are much more difficult to evaluate, particularly since quality is such a subjective characteristic. In general, a reasonable estimate of total crop loss from harvest to human consumption is about 33% (Kader and Rolle, 2004).

To minimize losses and to maximize both nutritional and economic value of horticultural commodities, it is essential to know characteristics for each product under consideration. In this chapter we will explore the many facets of harvesting and handling that have a significant impact on the postharvest life of horticultural products. In the following chapter (Chapter 16) we will consider the biology of postharvest physiology.

Quality, Maturity, and Ripeness

Quality is a subjective issue. Even though there are a number of attributes we consider that help to describe the quality of a commodity (Fig. 15.1), the end determination of whether or not a sample is good or poor quality is a matter of opinion. While every individual has his or her own idea of quality, an educated consumer is the best evaluator of quality. If the consumer does not like the product, it really doesn't matter what a physiologist says about the quality attributes observed.

Since quality is so subjective, quality standards have been adopted by many countries around the world to help maintain consumer confidence in the quality of horticultural commodities that they purchase. It is far beyond the scope of this book to examine these standards, however, a basic understanding of the parameters that go into setting up these guidelines makes understanding them less difficult.

Quality

The main components of quality include appearance (for all commodities), and texture and flavor for fruits, vegetables, herbs, and spices. The relative importance of each quality attribute depends on the individual assessing quality. A grower is often interested in the quantity and overall appearance of the commodity at harvest. A shipper or broker may be more concerned with firmness or stage of maturity that might affect longevity in storage or transit. The consumer is often most interested in taste and longevity after purchase. Even though everyone has their opinion of quality, in the long run, it is the consumer who ultimately determines the quality of an item since their assessment determines whether or not the item will be purchased again on their next visit to the market.

Appearance

Appearance can be broken down into size, shape, color, gloss, and freedom from defects and decay. Size, shape, color, and gloss are often determined by cultivar and management practices. Defects and decay may come from poor management decisions or pest problems but may also be an inherent physiological problem of the commodity. For example, russetting in Golden Delicious apple (*Malus domestica*) cultivars is an inherent defect in this strain of apple. On the other hand, bitter pit in apple is a visual defect that may be inherent to certain apple strains, but may be controlled with appropriate calcium management.

Texture

Texture includes such attributes such as firmness, crispness, juiciness, mealiness or toughness depending on the commodity. Texture is important in both

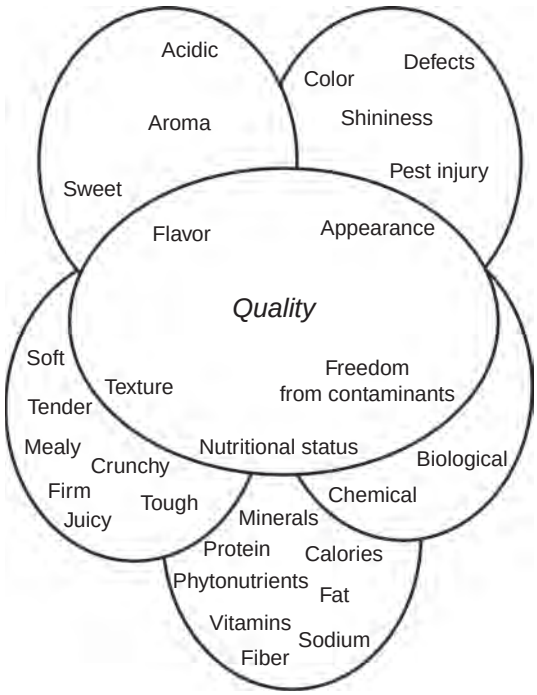


Fig. 15.1. Components of quality.

eating and shipping quality. Soft-textured commodities do not ship well and are often harvested at less than ideal maturity or ripeness in an effort to increase firmness and improve shipping ability at the expense of flavor.

Texture is not a single well-defined attribute, but rather a collection of intrinsic characteristics that help define quality that determines the acceptability of fruits and vegetables to the consumer (Abbott and Harker, 2004). Texture is often described as hard, soft, crisp, limp, mealy, melting, woolly, tough, leathery, gritty, stringy, dry, and juicy and is extremely subjective. Of these, only the firmness or lack thereof of the flesh is relatively easily measured and quantified. Firmness is often measured via the Magness-Taylor pressure test and the force needed to push a stainless steel tip of specified diameter a prescribed depth into the flesh of a fruit is reported as a value expressed in Newtons per square centimeter. Other tests such as a shear test (force required to cut tissues) or a compression test (force required to rupture cells in tissues) are used in research but not much in the produce industry.

Another reason it is often difficult to quantify texture is that each individual in a population of fruit varies, sometimes considerably, in texture due to the inherent nature of the quality. Almost constant changes at the cellular level make texture a changing attribute that is only a useful description at the time of evaluation and not for some time in the future during or after storage.

It is important to understand the nature of the commodity under evaluation in order to develop a good assessment of texture. Most commodities are composed of parenchyma cells which by nature are relatively soft and non-lignified. Cell walls (rich in cellulose) are joined together by the middle lamella, (rich in pectins and hemicellulose), which undergo varying degrees of decomposition and softening as a commodity matures, ripens, and senesces. The combination of cell wall strength, middle lamella composition and cellular turgidity all contribute to the perceived texture of the tissue in question. Some tissues have little intercellular air space, for example carrots (*Daucus carota*), while other tissues may have up to 25% air space, for example apples (*M. domestica*). Greater air space often leads to the perception of softer tissue. The hydration level of cell walls may affect the perceived juiciness of tissue. The perception of wooliness in apple and peach (*Prunus persica*) flesh may be due to cells separating at the middle lamella rather than cells rupturing during chewing, as occurs in crisp flesh (Harker *et al*., 1997).

Cooking often, but not always, softens tissue by degradation of pectin polymers combined with loss of turgor due to thermally induced membrane disruption. Some tissues, such as water chestnut (*Eleocharis dulcis*) or sugarbeets (*Beta vulgaris*), do not soften appreciably during cooking due to cell wall structure or due the presence of substances which enhance thermal textural stability (for example ferulic acid in water chestnuts) (Waldron *et al*., 1997).

Other factors also contribute to texture. For example different tissue zones in specific commodities such as: (i) the periderm, pericycle, and phloem parenchyma in carrots; (ii) the outer and inner pericarp and core in kiwifruit (*Actinidia deliciosa*); and (iii) the locules and gel of a tomato (*Solanum lycopersicum*). The different tissues differ in strength and must be taken into account when assessing texture. The epidermis of many fruits and vegetables is often thickened and may be covered in cutin which alters the texture. In addition the presence of collenchyma cells, for example in celery stalks (*Apium graveolens*) and pear flesh (*Pyrus* spp.), can have a profound effect on texture. Tough

fibers in the vascular tissue can cause stringiness in some commodities, for example pineapple (*Ananas comosus*) and asparagus (*Asparagus officinalis*).

Flavor

Flavor includes components such as sweetness, acidity, astringency, bitterness, aroma, and off-flavors. Flavor is an extremely subjective characteristic as human taste-bud sensitivity varies among individuals. Even though objective measurements are made on components of flavor, particularly sweetness (brix) and acidity (pH), evaluation by taste panels helps to define levels for measured components that produce acceptable flavor.

Flavor is governed mostly by sweetness and sourness that is perceived on the tongue in the parts per hundred range and is the component that receives the most attention. Aroma is a key component produced by volatiles that contributes to the perceived flavor and is often very difficult to separate from the other flavor components (Baldwin, 2004). Aroma is perceived in the nose at parts per billion levels. Other flavor components include bitterness, saltiness, and astringency. Sweetness is often the most important component of flavor for most produce. The brain takes the information gathered by the nose and tongue and synthesizes a perceived flavor that may change instantaneously. This makes flavor extremely subjective even though we can readily measure all of the components that are integrated into the flavor profile.

The sugars that play a key role in sweetness are: (i) fructose (sweetest); (ii) sucrose (less sweet); and (iii) glucose (least sweet). Soluble solids content (SSC) is easily measured using a refractometer, however, the perceived sweetness is not always linearly related to SSC and may also be affected by the levels of the different sugars in the sample. Citric, tartaric and malic acids are some of the key organic acids that impart sourness to products. Some commodities such as banana (*Musa* spp.) and melons (*Cucumis melo*) have little acidity. Acidity is often assessed by titration (titratable acidity, TA) or by simply measuring pH. The perceived sourness is usually best reflected by a ratio of SSC to TA or pH, rather than TA or pH measurements alone.

Aromatic compounds are usually released during cell disruption during eating or food preparation when enzymes and substrates that were previously compartmentalized are allowed to interact (Buttery, 1993). Further release of volatiles often accompanies cooking. Measuring specific compounds is difficult and time consuming and measured levels may not necessarily relate in a consistent fashion to a perceived flavor or aroma profile.

Often taste test panels can provide a better quality rating than measuring flavor or aroma components. Flavor of horticultural products is often evaluated using trained test panels to select promising new cultivars followed by consumer taste tests to determine acceptance of previous selections. Other quality attributes are usually evaluated along with flavor. Often a simple three-point scale with the options of 'like', 'no opinion' and 'dislike' for each quality attribute is sufficient to identify acceptable or preferred samples.

Genetics is the most crucial element in determining flavor (Baldwin *et al*., 1992) with other factors such as pre-harvest environment, cultural practices, harvest maturity, and postharvest handling having less of a role. Thus cultivar selection is crucial in flavor considerations as the genetics of the cultivar provide the base which production factors may act on to modify flavor quality.

Nutritional status

An increasingly important component to quality of edible horticultural products is their positive contribution to human health. Fruits, nuts, and vegetables provide vitamins C, A, thiamine (B1), niacin (B3), pyroxidine (B6), folacin (folic acid or folate) (B9), and E, as well as minerals and dietary fiber. Most commodities are low in fat and cholesterol and have a reasonable caloric value. Estimates of significant plant contributions of vitamins and minerals to the average US diet are 91% of vitamin C, 48% of vitamin A, 30% of folacin, 27% of vitamin B6, 17% of thiamine, 15% of niacin, 16% of magnesium, and 19% of iron (Kader *et al*., 2004). Legume vegetables, potatoes (*Solanum tuberosum*), and nuts contribute close to 5% of the protein consumed in the average US diet. Nuts are also rich in essential fatty acids. Many commodities supply antioxidants, and compounds that are anti-carcinogenic, and that help alleviate problems caused by diabetes and heart disease.

While genetics plays a major role in which beneficial compounds are produced by specific plants, temperatures favor sugar and vitamin C synthesis while β-carotene (vitamin A) synthesis is promoted by more moderate temperatures (15–21°C). Warm-season crops produce more B vitamins at warmer

temperatures (27–30°C) while cool-season crops produce more B vitamins at lower temperatures (10–15°C). The production of B vitamins is not affected by light levels while vitamin C increases and vitamin A decreases under high light levels (Gross, 1991).

The nutritional contribution of horticultural products often decreases after harvest but the rate of loss can be minimized with proper postharvest handling with each commodity (Lee and Kader, 2000). Water-soluble nutrients are often lost when cooking water is discarded rather than consumed while fat-soluble nutrients may be stabilized or their levels may be enhanced with cooking. The human health benefits of horticultural products are discussed in depth in Chapter 17, this volume.

Freedom from contaminants

Another important quality attribute of many horticultural commodities is freedom from chemical or biological contaminants. Chemical contaminants can include natural compounds such as glycoalkaloids in potatoes (*S. tuberosum*) or fungal and bacterial toxins as well as heavy metal contaminants. Pesticide residues are also an important contaminant often found on commodities and it is often the most important concern for many consumers (Kader and Rolle, 2004) even though microbial contamination especially by *Salmonella, Listeria, Escherichia coli* and others is considered more harmful by many authorities.

BIOLOGICAL CONTAMINANTS One of the major biological hazards for humans consuming fresh produce is contamination from microorganisms including bacteria, parasites, and viruses. While many microorganisms are beneficial to humans (they give us bread, wine, cheese, and sauerkraut), the wrong organism in the wrong place at the wrong time can cause serious, sometimes fatal food-borne illnesses. Either the organism itself or toxic metabolites can cause harm.

Some bacteria such as *Clostridium botulinum, Bacillus cereus* and *Listeria monocytogenes* are found in the soil and can easily contaminate produce. Other bacteria such as *Salmonella, Shigella*, pathogenic *E. coli*, and *Campylobacter* live in human and animal intestines and can contaminate produce through: (i) infiltration of sewage into production fields; (ii) irrigation with contaminated water; (iii) animals wandering in production fields; or (iv) using incompletely composted animal manure on production fields. Handling of produce by contaminated individuals can introduce pathogens at any stage along the production path (Beuchat, 1998). In some cases, more than a million cells of the pathogen per gram of food are required to cause illness and in others, such as with *Shigella*, only ten cells per gram are needed to cause illness. The bottom line is that all steps must be taken to prevent bacterial contamination of produce and to prevent growth of contaminants that might already be present through handling and storage under proper conditions. This is especially important since much of the produce consumed by humans is consumed raw.

Parasites such as *Cryptosporidium, Cyclospora, Giardia, Entamoeba, Toxoplasma, Sarcocystis, Isospora*, and nematodes can be transmitted in infected produce after contamination by an infected individual, water contaminated with fecal material, or animals wandering in the production field. These parasites can cause severe gastrointestinal distress and even death. Viruses including hepatitis A virus, Norwalk virus, rotaviruses, astroviruses, enteroviruses (polioviruses, echoviruses, and coxsackie viruses), parvoviruses, adenoviruses, and coronaviruses, can be transmitted through infected produce.

Control strategies for minimizing contamination include general good agricultural practices like: (i) irrigating with clean water; (ii) using only properly composted manure; and (iii) ensuring worker hygiene and proper sanitation during handling and storage. The most important control strategy is maintaining worker hygiene (Beuchat, 1998).

CHEMICAL CONTAMINANTS Chemical contamination of produce may occur naturally or may arise from contamination along the production chain. Some common potential contaminants include polychlorinated biphenyls (PCBs), lubricants, cleaners, pesticides, fertilizers, sanitizers, antibiotics, lead from packaging materials, and heavy metals from contaminated soil. Some naturally occurring chemical contaminants might include mycotoxins like aflatoxin, mushroom toxins, alkaloids, and phytohemagglutinin, a toxin produced in kidney beans (*Phaseolus vulgaris*). Chemical contaminants may cause acute or chronic illness.

PHYSICAL CONTAMINANTS Many physical contaminants could find their way into fresh produce and pose a serious hazard. Some examples of common

foreign materials found in fresh produce include glass fragments, wood shards, stones, plastic packaging material, staples, jewelry, and hair clips. Most incidents of contamination with foreign objects are accidental and hard to prevent. Thorough inspection of produce during packaging helps reduce this type of contamination.

Harvesting

Harvesting is the removal of the commodity from the parent plant or the removal of the entire plant from the production field if it is the economic commodity. Many products can be harvested at various stages of development. However, in general there are standards for harvesting most commodities based on size and/or stage of development. A major stage of development, particularly in fruit is maturity.

Maturity is a term often associated with commodities in which the harvested portion is a botanical fruit. The fruit is mature if it has reached the stage of development just prior to ripening and would continue development if removed from the plant. Immature fruit will not continue to develop. Fruit is often divided into two groups: (i) those that will not ripen once removed from the plant; and (ii) those that will ripen if harvested mature. A ripe fruit is ready to eat. Fruit that will not ripen once harvested include cane berries (*Rubus* spp., *Ribes* spp.), cherry (*Prunus* spp.), citrus fruits (*Citrus* spp.), grape (*Vitis* spp.), lychee (*Litchi chinensis*), pineapple (*Ananas comosus*), pomegranate (*Punica granatum*), strawberry (*Fragaria* × *ananassa*), and tamarillo (*Solanum betaceum*). Fruit that can ripen if harvested mature and exposed to the proper environment include apple (*M. domestica*), apricot (*Prunus armeniaca*), avocado (*Persea americana*), banana (*Musa* spp.), cherimoya (*Annona cherimola*), guava (*Psidium* spp.), kiwifruit (*A. deliciosa*), mango (*Mangifera indica*), nectarine (*P. persica*), papaya (*Carica papaya*), passion fruit (*Passiflora edulis*), pear (*Pyrus* spp.), peach (*P. persica*), persimmon (*Diospyros* spp.), plum (*Prunus* spp.), quince (*Cydonia oblonga*), sapodilla (*Manilkara zapota*), tomato (*S. lycopersicum*), cantaloupe (*C. melo*), and watermelon (*Citrullus lanatus*). Immature fruit will not ripen once harvested. Most fruit attain best eating quality if allowed to ripen on the plant. However, to enable long-distance shipping, many commodities are harvested mature but not ripe.

Fruit are often categorized as climacteric or non-climacteric. Climacteric fruit are those fruit that can be harvested mature and will ripen after harvest (the second group from above). Non-climacteric fruit (the first group) will not ripen once harvested. Non-climacteric fruit have a low, relatively constant rate of ethylene production as it proceeds from maturity through ripeness and senescence. In addition, the rate of CO_2 is relatively constant through the ripening process. Climacteric fruit are characterized by a rapid (sometimes 1000-fold) increase in ethylene production as ripening is initiated. This rapid increase in ethylene is closely followed by a sharp increase in CO_2 production. Ethylene production is an autocatalytic process: ethylene induces ethylene production. Regulation of ethylene production especially in climacteric species is a focal point of postharvest physiology (see Chapter 16, this volume). Climacteric fruit can be induced to ripen by exposing them to ethylene.

Many vegetables are harvested immature. This includes leafy vegetables and vegetables where the harvested portion is immature fruit. For example cucumbers (*Cucumis sativus*), sweet corn (*Zea mays*), green beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), and okra (*Abelmoschus esculentus*). Most of these vegetables reach optimum eating quality prior to maturity. Delayed harvest of many vegetables leads to inferior quality.

Maturity indices

Maturity or harvest indices are visual, physical, and chemical attributes that allow the grower to determine the best time to harvest a particular commodity (Fig. 15.2). These indices are a compromise between a stage that is best for eating and a stage that is best for marketing. Maturity indices have been developed for leafy vegetables, root vegetables, 'fruit' vegetables, and fruit crops. In many instances several maturity indices are combined when evaluating a crop and its suitability for harvesting.

Physical attributes

The most common harvest index for many commodities is size. The worldwide produce industry has established a standard size for each commodity at harvest. This may include a minimum and maximum size as well as different grading categories for different sizes. Size is usually length or diameter or a combination of the two. Some commodities such as

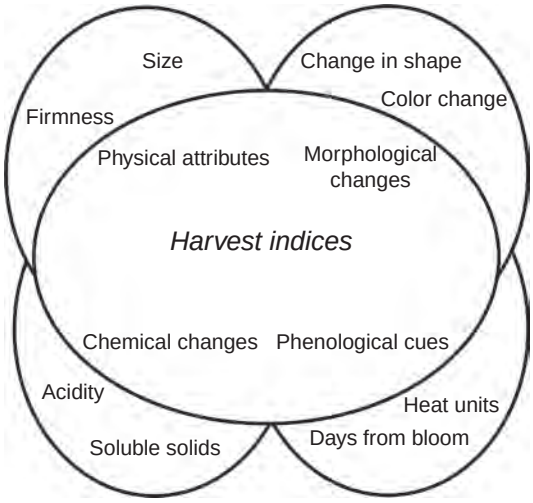


Fig. 15.2. General harvest indices for horticultural commodities.

banana (*Musa* spp.) and cucumber (*C. sativus*) change from angular to nearly round as they reach maturity and this can be used as a signal to harvest.

Fruit firmness is another harvest index, easily measured with a penetrometer. A penetrometer is a small, usually hand-held device that consists of a gauge to which a slender compression cylinder is attached. Different diameter tips are available that are attached to the compression cylinder according to the crop under consideration. The tip of the cylinder is marked so that the operator knows the depth to which the tip should be inserted into the flesh. Normally the skin of the fruit is removed and the tip of the compression cylinder is forced into the flesh to the line on the tip. The force needed to insert the tip registers on the gauge and is recorded as values expressed in Newtons·per square centimeter. The old method of reporting pressure in kilograms per square centimeter is no longer acceptable. A consistent methodology should be developed so that the tip is pressed into the flesh in a similar manner among the fruit being tested. Several readings on opposite sides of the fruit are often taken to better estimate firmness.

Morphological changes

Morphological changes are often used as maturity indices. These include the development of an abscission zone at the point of fruit attachment to the vine in muskmelons (*C. melo*) and curling and drying of the tendril adjacent to the point of fruit attachment to the vine in watermelon (*C. lanatus*). The development of a waxy layer on the epidermis of plums (*Prunus* spp.), grapes (*Vitis* spp.), and honeydew melons (*C. melo*) can be used to determine time of harvest. Netting develops on muskmelons as they approach maturity. Gel-like material forms around tomato (*S. lycopersicum*) seeds as the fruit begins to mature.

Internal or external fruit color is used as a maturity index for some fruits such as apricot (*P. armeniaca*), nectarine (*P. persica*), persimmon (*Diospyros* spp.), strawberry (*Fragaria* × *ananassa*), peach (*P. persica*), plum (*Prunus* spp.), and raspberry (*Rubus* spp.). Color is a fine index of visual quality but has little to do with flavor quality. The percentage juice by volume is a maturity index for lemon (*Citrus* × *limon*). Again, this single attribute confers nothing concerning the flavor quality of the juice. Fruit tenderness is used to determine optimum harvest for peas (*P. sativum*). Solidity or firmness of developing heads is used to determine when to harvest head lettuce (*Lactuca sativa*) and cabbage (*Brassica oleracea* Capitata Group).

Chemical changes

SSC (soluble solids content) is another index of maturity associated with the sugar content of produce and is reported as a percentage or as degrees brix (°BX). Some crops must reach a minimal level of SSC before they can be harvested for sale. In many cases higher SSC is associated with higher quality. Acidity is often measured as well as pH or TA (titratable acidity) and a ratio of SSC to pH or TA can provide a reasonable estimate of flavor quality. Persimmons (*Diospyros* spp.) are evaluated for astringency as a harvest index.

Some crops such as apples (*M. domestica*) utilize a starch-iodine test to reflect the amount of starch contained in the product that potentially will be converted to sugars as the product matures and ripens. A solution of tincture of iodine is applied to the cut surface of the produce. Any starch in the tissue will stain black and the relative amount of starch in the tissue can be ascertained by comparing the degree of staining of the sample to standardized stain color charts.

Phenological development

Days from blossom or days from seeding are often used to estimate harvest date. In general, this

method will provide a reasonable estimate of when the crop should be harvested. A better estimate can be obtained if temperature data is available to calculate heat units and the number of heat units required for crop development and maturation is available from references.

Harvest

Harvesting is a critical stage in any horticultural production chain. Once produce is harvested, quality does not improve, it can only be maintained. Every effort must be made to: (i) harvest during the right time of the day to maintain quality; (ii) harvest at the appropriate stage of development; and (iii) harvest only produce that has the potential to hold up during storage, shipping, and sale. Once harvested, the commodity must be handled to maximize shelf life. A properly trained, skilled, hygienic harvest team is a worthwhile investment for any grower as commodity handling from harvest through to consumption is critical for maintaining quality. Pickers and supervisors who know what they are doing and why they are doing it go a long way to ensure success.

Most commodities should be harvested during the coolest part of the day, usually early morning. In some regions this may present a problem since produce may be wet from dew. Many commodities should not be harvested wet to minimize the chances of contamination and decay during storage. Harvesting when it is cool is tied to the product's respiration rate.

Respiration uses substrates from the commodity to provide the energy required for metabolic maintenance. Once harvested, the commodity is no longer manufacturing substrates, so any that are used postharvest are from those stored in the commodity. The faster that stored substrates are used, the faster the product deteriorates. By harvesting when the temperature is cool, respiration is minimized as much as possible. In addition, the amount of field heat that must be removed before storage is decreased.

Most produce is harvested based on harvest indices specific to each crop. Besides knowing when to harvest, it is important to know how to harvest. One major rule of thumb is to be as gentle as possible with produce during the entire harvesting, sorting, cleaning, packing, storing, shipping, and selling chain of events to minimize mechanical damage to the product. Damage immediately decreases the quality and therefore value of the product and renders it more susceptible to disease-causing organisms and physiological decay due to the sound-induced ethylene produced. All harvesting tools and containers should be disinfected daily with either a chlorine- or hydrogen-dioxide-based solution and thoroughly rinsed. Cutting tools should be sharp and disinfected and workers should be trained in their proper use. Vehicles and work areas should be kept clean of crop debris and trash. Produce should be kept as cool as possible and placed in cold storage (when appropriate) as soon as possible.

Grading and culling should start in the field at harvest with only high quality marketable produce selected and harvested. Minimal culling should be required at the packing area. Many products are field packed as they are harvested, minimizing the number of times the product is handled. Each handling costs time and money and presents the opportunity for mechanical damage and bruising. All workers assisting in harvesting and packaging must follow good hygiene and sanitation practices. Field containers should not come in contact with soil and should be free of dirt and other contaminants. Field sanitation is especially important for crops that are not washed before storage, such as grapes (*Vitis* spp.) and strawberries (*Fragaria* × *ananassa*).

Field heat removal

One of the most important things to do at harvest to maximize storage life and quality is to remove as much field heat as possible from the commodity as quickly as possible. Cooling to remove field heat may occur in mobile field units or at the packing or storage facility. Removing field heat helps prolong storage life and quality by: (i) reducing respiration and the production of ethylene; (ii) reducing water loss from the product (which can be substantial during storage); and (iii) reducing the growth of harmful pathogens in or on the product.

Heat is removed from produce by two mechanisms: (i) conduction; and (ii) convection. Conduction removes heat from a commodity by transferring heat from within a product to its coldest surface. With convection, heat is removed by carrying it away in a medium such as air or water. There are several methods of removing field heat from produce and all must minimize opportunities for produce contamination. The major concern is

contamination by microorganisms. Heat is usually removed by air either by storing produce in a cold room or by forced air cooling, or with water via hydrocooling or icing of produce.

The simplest heat removal is accomplished by placing produce in a cold room. Cooling occurs very slowly and may be unacceptably slow for some commodities. Cooling can be hastened by forcing cooled air over the product and through the storage containers. The source region for the air used in forced air cooling must be clean and free of possible contaminants and filters should be checked and cleaned regularly. Using water (hydrocooling) or ice as the cooling medium rather than air hastens cooling significantly, however, the chances of contamination also increase. Water used for cooling must be replaced at least daily and ice must be made from contaminant-free, chlorinated, potable water and stored in sanitary conditions.

Another method of cooling certain produce, such as root crops, broccoli, and Brussels sprouts, is by placing ice or 'liquid ice' (60% ice, 40% water) in direct contact with the produce. Regular ice contains large amounts of air which reduces direct produce-to-ice contact and results in slower, less efficient cooling. Liquid ice creates much better contact with greater rates of cooling.

Vacuum cooling is accomplished by placing the produce in an airtight steel container and applying a vacuum to it. The vacuum causes water in the produce to vaporize and thereby cool the produce. Faster cooling is achieved with increased exposure of produce surfaces, allowing more water to vaporize more quickly. Produce usually cooled this way includes iceberg lettuce (*L. sativa*), celery (*A. graveolens*), cauliflower (*B. oleracea* Botrytis Group), sweet corn (*Z. mays*), carrots (*D. carota*), and sweet peppers (*Capsicum annuum*). The major disadvantage of this method is that 1% of the produce weight is lost during cooling as water is removed by vaporization for each 5°C drop in produce temperature (Holdsworth, 1985). Hydro-vacuum cooling is a modification that showers the produce with water during the cooling process to reduce the water lost from produce during cooling.

Postharvest water quality

Water is used for many postharvest operations including: (i) quick washes to remove field dirt; (ii) to minimize bruising in dump tanks; (iii) to remove field heat; (iv) to prepare waxes or fungicide dips; and (v) for heat treatments to remove insect pests. No matter what its use, water must be clean, potable and kept that way during the process in which it is used. Keep in mind that clean, potable water can quickly become contaminated by contaminated produce placed in it. Even produce that appears to be clean can harbor considerable contaminants, especially in warm wet weather.

Chlorine is often added as a sanitizer to water used for postharvest processing. It is most often added as sodium hypochlorite, calcium hypochlorite, or liquid chlorine. As chlorine reacts with organic matter from the produce during postharvest processing, it loses its effectiveness. Therefore the free chlorine level of the water must be regularly monitored (at least hourly) and maintained at a level of at least 200 ppm. The water must be replaced on a daily basis even with chlorine treatment and local environmental guidelines must be followed regarding disposal of the wastewater.

One important consideration when using water during postharvest processing is that in some commodities, water and any microbial or chemical contaminant can be actively absorbed into the product during processing, especially if the produce temperature is much higher than the water temperature. When warm apples (*M. domestica*), celery (*A. graveolens*), mangoes (*M. indica*) or tomatoes (*S. lycopersicum*) are placed into cold water, a water pressure differential develops that creates a suction effect that leads to infiltration of water into the produce. Any contaminant that may be in the water is drawn into the produce, away from any possible effect of sanitizers added later. This stresses the importance of water monitoring for effective sanitizer concentration during processing to reduce the possibility of contamination (Sargent *et al*., 2007). To avoid this potential problem, the temperature of any water used for washing should be 5°C above the temperature of the flesh of the commodity and cooling should be accomplished using forced air.

Cleaning produce

During postharvest handling, one major objective, besides removal of field heat, is to clean produce for packaging and sale while avoiding bruising and contamination. Most produce should be cleaned of major debris during harvest. Additionally, most produce is relatively free of harmful microorganisms at harvest and only becomes contaminated

with harmful pathogens during harvest and post-harvest processing. To minimize contamination, produce that can tolerate water after harvest is often sanitized during the packaging process. Some produce such as raspberries (*Rubus* spp.), strawberries (*Fragaria* × *ananassa*) and grapes (*Vitis* spp.) cannot tolerate water after harvest and thus cannot be sanitized.

After harvest any remaining surface soil or debris is removed with brushes or forced air, depending on the product's ability to tolerate each. In products that can tolerate water after harvest, a thorough spray wash with chlorinated water is utilized. Food-grade detergents might be used if produce is extremely dirty. A second spray wash may be needed and several spray washes are usually more effective than one long-soaking wash. Again the main concern is to use chlorinated, potable water for the washes. Soft produce is normally washed using only water sprays, while other products may be washed in flumes or with brushes. If brushes are used, they must be routinely cleaned and sanitized.

After cleaning, a sanitizing step is often used, followed by a final wash. It is important to remember that sanitizing reduces the level of pathogens on produce and does not completely eliminate them. Only sterilization, a much more rigorous process, can completely eliminate pathogens. Sterilization is usually accomplished by heat. A chlorine solution is usually used for sanitizing. Immersing produce in a chlorine solution with 50–200 ppm free chlorine for 1 to 2 min is usually sufficient for sanitizing. Chlorine solutions contain molecules of hypochlorous acid (HOCl) and its ions H^+ and OCl^-. The HOCl is the toxic component that kills microorganisms and the equilibrium between HOCl and H^+/OCl^- is determined by the pH of the solution. A lower pH favors the lethal HOCl, but can quickly corrode metal sanitizing equipment. Generally, a pH of 6.0–7.5 at 20°C is a good compromise as it allows enough of the HOCl to exist to sanitize the produce yet at the same time minimize the corrosion of equipment. Other sanitizing agents occasionally used include chlorine dioxide, bromide, iodine, trisodium phosphate, quaternary ammonium compounds, organic acids, hydrogen peroxide, peracetic acid, and ozone.

Irradiation is often cited as a controversial method of produce sanitation. To kill most pathogens, most produce would require irradiation doses that are too high and lead to softening and off-flavor development (Farkas *et al*., 1997).

Sanitation during packing, storage, and transportation

The major concern during packing, storage, and transportation of produce should once again be sanitation and prevention of contamination by harmful microorganisms while maintaining product quality. Worker hygiene and sanitation must be maintained during these stages in the handling chain and all facilities utilized during these stages, including vehicles used for transportation, must be kept clean, safe, and sanitary.

Packaging

How commodities are packaged for storage, transportation, and sale has a marked effect on how well their quality holds up during the process. Some commodities such as strawberries (*Fragaria* × *ananassa*) and raspberries (*Rubus* spp.) are harvested into the containers in which they will be stored, transported, and marketed. Other commodities such as broccoli and kale (*Brassica oleracea*) are harvested into the containers that they will be stored and shipped in, then repackaged for marketing. No matter which types of packaging are used, they all seek to maximize product longevity while maintaining quality.

The major factors that govern product stability during the marketing chain are: (i) water loss; (ii) respiration (loss of sugars); and (iii) senescence accelerated by ethylene. These factors will be investigated more thoroughly in Chapter 16, this volume. However, it makes sense to briefly consider them here.

Water loss

Water loss is important during storage, transport, and marketing from two perspectives. First, since many commodities are sold by weight, water loss is weight loss which translates into a loss in value of the product. Secondly, water loss is usually associated with a loss of textural quality of the product. Consider wilted lettuce or a head of broccoli that has dehydrated somewhat.

Most products are stored in some sort of water-retaining package after harvest. This may be a waxed box, a plastic-lined cardboard box, plastic bags, or clamshell containers. Containers are often vented to prevent the build up of excessive moisture on the produce and to allow CO_2 and ethylene

to escape while maintaining the minimum O_2 requirements for maintenance respiration.

Modified atmosphere packaging (MAP)

Quality of many commodities is often maintained from harvest to market using MAP (Mir and Beaudry, 2004). MAP was originally developed in the 1940s for apple marketing. The packaging maintains an internal predetermined, commodity-specific atmosphere which is generated by the product itself. In general a high CO_2 level coupled with a low O_2 level is desired to reduce the metabolism of the produce and any potentially harmful decay organisms that may be present. MAP films are often impermeable to water, thus they retain moisture so important in product quality. The films also isolate the product from external pathogens and contaminants. A major drawback to MAP is the potential development of fermentation products and off-flavors due to excessively low O_2 levels or excessively high CO_2 levels. Commodities vary significantly in the optimum concentrations of O_2 and CO_2 and ranges for successful storage are available in Mir and Beaudry (2004).

Containers used for MAP achieve the desired gas concentrations through selective permeability, perforations in the film, or a combination of the two. Different films are available to achieve desired atmospheres. The permeability of most films changes over time and responds to changes in humidity and temperature following physical chemistry laws. The gas composition inside the container can be controlled by altering the external environment. The response of the commodity to the container's internal atmosphere can vary with species, cultivar, cultural practices, harvest method, and postharvest handling. Flushing the container with a specific gas mixture is often incorporated into the postharvest plan to achieve optimum initial internal conditions for the commodity.

In continuous films where gases must diffuse through the film, a steady state of O_2 and CO_2 is achieved only if the respiration rate is constant. The rates of O_2 uptake and CO_2 production must also be equal to their rate of diffusion through the film, which is driven by the gradients of the two gases across the film. In perforated films, gas movement is a combination of movement through the perforations and diffusion through the film. Gas movement through the perforations is usually much greater than diffusion through the film and this type of film is usually used for products having a high demand for O_2. The permeability of continuous film packaging increases with temperature while permeability of perforated packaging is not sensitive to changes in temperature.

The internal atmosphere of MAP containers is mainly a function of commodity, film characteristics, and temperature. In order to account for changes in temperature a commodity is likely to experience along the marketing chain, companies must select a strategy to maximize storage life and maintain quality. Most companies select packages based on the highest temperatures normally encountered along the marketing chain and as much as possible maintain the product at the lowest acceptable temperature. This approach accounts for the fact that temperature has more of an effect on maintaining product quality than internal-package gas concentrations (Kays, 1997). Most packages are designed to maintain internal O_2 concentrations well above the lower limit set for a specific commodity to essentially guarantee aerobic conditions at all times. Anaerobic conditions at any time would lead to rapid quality deterioration.

Condensation inside the packaging can be a problem if the commodity experiences moderate to rapid temperature changes. Condensation is often unsightly and may contribute to product decay. Films are often treated with different chemicals during manufacturing that help reduce condensation or that result in ultra-fine droplets that are nearly invisible when condensation does occur.

Ethylene is the potent, autocatalytic hormone that can accelerate or induce ripening and senescence in many commodities. Once ethylene initiates its effects, the senescing process cannot be stopped. Small sachets filled with potassium-permanganate-impregnated zeolite are often placed in the package as an ethylene scrubber. Zeolite is a mineral that is known for its odor-absorbing properties, thus these sachets also help reduce any off-odors as well. The potassium permanganate reacts with ethylene to form CO_2 and water.

Transportation

Adequate transportation from harvest to market is an integral component of successful horticultural production. An excellent reference for transportation of horticultural commodities is Ashby (2006). If the farm is a pick-your-own facility, no transportation at all is required. If products are sold on

farm only transport from the field to an on-farm grading, packing, storage, and sales facility is required. In many cases, longer distance transport begins on the farm via truck, followed by transport to a main distribution center via truck, rail, ship, or air, then finally from the distribution center via truck to the marketplace. In all cases the two main concerns for any horticultural product during short- and long-distance transport are sanitation and temperature.

Temperature

Sanitation was reviewed earlier in this chapter. All vehicles and facilities involved in transport must be maintained in a clean, sanitary condition. Product temperature must be maintained as close to that recommended for any particular commodity to ensure longevity and quality. Field heat must be removed and the product transported, stored, and marketed at the appropriate temperature.

While some commodities can be shipped at ambient temperatures, most products must be maintained at an acceptable temperature by refrigeration during transport. Any deviation, either high or low, from the desired temperature can lead to a decrease in storage life and product quality. These deviations are additive in that while any individual deviation may seem inconsequential, the net effect on the product is one that is the sum of each individual infraction (Ashby, 2006). An important consideration regarding the response of commodities to temperature infractions is that injury they cause may not show up until the consumer has already purchased them and is preparing them for consumption. For example, sugar depletion in sweet corn (*Z. mays*) due to handling at warmer than required temperatures is not visible and is normally not detected until consumption. Other commodities may fail to ripen properly, have internal browning, or external pitting due to temperature extremes.

Temperature control during transport is often difficult for a number of reasons. The external environment is usually in constant flux as the transportation vehicle moves along. Heat from the vehicle and any heat entering the transport vehicle from the outside via conduction through the transport vessel or via infiltration through small cracks and holes in the vessel must be removed. Any excess heat in the commodity that was not previously removed and heat generated by product respiration must also be removed during transit. Finally, several different commodities each with separate temperature optima are often transported together.

Most transport vehicles utilize mechanical refrigeration for cooling. Mechanical cooling systems move heat from inside the transport vessel and release it outside the vessel via a circulating refrigerant. The refrigerant absorbs heat in an evaporator coil located inside the cargo container, is circulated to the condenser coil on the outside of the container to release the heat, then re-circulated via a gasoline, diesel or electric compressor back to the evaporator to absorb more heat. Most refrigeration units are controlled by a microprocessor which constantly monitors temperature and performance. Container and commodity temperature as well as transport location are often monitored via satellite uplink. Some commodities are topped with crushed ice before transport to: (i) reduce their temperature; (ii) decrease the refrigeration requirement; and (iii) reduce water loss during transit. Depending on the length of the trip and container temperature, ice may need to be replenished during transport.

When commodities are shipped during winter months in cold climates, the transport container may require heating rather than cooling to maintain an appropriate temperature. The potential for both freezing and chilling injury must be considered during transit and appropriate measures taken to ensure that neither occurs.

Humidity

Most horticultural commodities should be maintained at 85–95% RH. One of the detrimental aspects of using mechanical refrigeration systems is that they inherently remove water from the air around them, causing water to be removed from the commodity via evaporation. Water loss can be minimized by: (i) transporting only pre-cooled product which minimizes the temperature differential between the product and the air in the transport vehicle, which minimizes the water lost during cooling; (ii) maintaining a coil temperature of just a few degrees below the desired air temperature; (iii) waxing the product; (iv) utilizing semi-permeable containers or wraps; and (v) installing a humidity controller in the cargo vessel.

Controlled or modified atmospheres

Many commodities that are shipped long distances are shipped under controlled or modified

atmospheres. The atmosphere inside the shipping vessel is modified with one or more gases (usually O_2, CO_2, N_2) to reduce product metabolism and retard growth of organisms that may cause spoilage. Controlled atmospheres are constantly monitored to maintain a specific gas composition while modified atmospheres are initially established at a specific composition but not monitored during transit. Modified atmospheres may also be established within containers used for retail marketing, such as bags or clamshells, rather than within the entire transit vessel. Nearly every commodity has a specific ideal atmospheric composition for storage and transit. The ideal composition of one commodity may adversely affect another, thus caution must be taken when transporting mixed loads under controlled or modified atmospheres. Additionally, temperature and humidity may modify the commodity response to the controlled or modified atmosphere.

Ethylene

Consideration should also be given to ethylene generation or sensitivity during storage. Some commodities produce large quantities of ethylene during storage and transit while others produce little or none and may be harmed by exposure to the ethylene produced by other commodities in a mixed load. Ethylene scrubbers can help reduce this problem.

Odors

In addition, some commodities have a tendency to absorb odors and should not be shipped with those producing a strong odor. For example, apples (*M. domestica*), citrus (*Citrus* spp.), onions (*Allium cepa*), and pineapples (*Ananas comosus*) often give off considerable odors. Some commodities such as apples can both give off and absorb odors (Ashby, 2006). Some transport combinations to avoid include apples or pears (*Pyrus* spp.) with potatoes (*S. tuberosum*), as the apples or pears will often acquire an 'earthy' taste if shipped with potatoes. Also apples and pears should never be shipped with celery (*A. graveolens*), cabbage (*B. oleracea* Capitata Group), onions (*A. cepa*), or carrots (*D. carota*). Citrus (*Citrus* spp.) should never be transported with onions, cabbage, cauliflower (*B. oleracea* Botrytis Group), broccoli (*B. oleracea* Italica Group), or any other strongly scented commodity.

Marketing

Once a commodity reaches its destination market, its quality and safety must be maintained via good attention to sanitation and storage conditions, most notably temperature, humidity, and ethylene levels. Some products may need to be repacked or packaged from bulk shipping containers before sale. Products should not be excessively handled during repackaging or sale, as significant bruising and quality deterioration may result. Certain commodities such as avocados, bananas, and tomatoes may require pre-sale ripening.

Food Laws and International Regulations

Food safety and security is an important global issue. Various laws and regulations within countries attempt to ensure that all food offered for sale is safe and fit for human consumption and produced, packaged, stored, and transported under sanitary conditions. Many countries also require adequate labeling of food products to provide information regarding content, country of origin, and nutritional content. Since it is far beyond the scope of this text to review the food regulations of nations individually, it is appropriate to review the international codes for food safety adopted by most countries.

Human, animal and plant health standards are necessary to ensure a safe and secure food supply and to help prevent the spread of diseases and pests from one region of the world to another. In 1994 the World Trade Organization (WTO) was formed and established two binding agreements focused on agriculture: (i) the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS); and (ii) the Agreement on Technical Barriers to Trade (TBT) (JIFSAN *et al.*, 2002). The SPS agreement seeks to ensure that measures established by governments to protect human, animal and plant health in the agricultural sector are consistent with attempts to prevent trade discrimination among adhering countries. The agreement also requires that participating countries adhere to guidelines adopted by the Food and Agriculture Organization (FAO)/World Health Organization (WHO) Codex Alimentarius Commission (CAC). Countries may, however, employ stricter guidelines when justified. Thus it provides a minimum stand by which countries must abide regarding human, animal and

plant health in an agricultural context. The SPS agreement encompasses all food hygiene and safety measures including pesticides and other agricultural chemicals, as well as plant quarantine measures as established by the International Plant Protection Convention (IPPC). Animal health standards are set by the International Office of Epizootics.

The TBT agreement prevents localized technical requirements from becoming barriers to international trade and includes many measures to protect consumers from deception and fraud. For example, the TBT agreement covers standardization of quality and labeling considerations as related to international agricultural products and trade.

Codex Alimentarius literally means 'food code' and it is a set of food standards set up by the CAC that countries can adapt to their local food regulations to keep them in line with international trade. The main benefit of the code is that it provides reasonable assurances that food products are produced in a safe and hygienic manner and that they are nutritious and provide acceptable health protection. The specific codes that have been adopted by he CAC can be found online at: http://www.codexalimentarius.org/standards/en/.

16 Postharvest Physiology

In the previous chapter some of the major events between harvest and marketing were reviewed. In this chapter, the major physiological events that occur postharvest are considered.

Physiology of Horticultural Products After Harvest

Respiration

All horticultural commodities begin to senesce once harvested. After harvest, all metabolism is catabolic since the commodity no longer photosynthesizes or is attached to a photosynthesizing plant. Some compounds may be synthesized (anabolic metabolism), but only at the expense of some other compound already contained in the harvested commodity. Another way of looking at it is that the total energy status of the commodity will never be higher than it is at harvest. At harvest it is removed from its source of energy, photosynthesis.

Probably the most important physiological process occurring after harvest is respiration, the controlled release of energy within a living organism. In respiration, compounds, usually sugars, combine with O_2 in a precise, controlled manner to release energy, producing CO_2, and water. This energy released in respiration is utilized in other metabolic activities of the commodity or lost as heat. The heat given off by respiring commodities is often called vital heat and is important in estimating refrigeration requirements. Since any commodity has a specific and limited quantity of reserves in it for energy production after harvest, how rapidly this store is depleted is the major factor determining postharvest life. Postharvest life is the amount of time a product is useful to the consumer. This might be the amount of time a peach is suitable for consumption or how long a cut flower is pleasing to look at. A major focus of postharvest physiology is maximizing the useful postharvest life of a product while ensuring acceptable quality usually by effectively managing the storage environment. A long storage life is useless without quality.

In general, commodities with a high respiration rate have a low storage life and those with a low respiration rate have a long storage life. Respiration affects the levels of many of the compounds responsible for perceived quality and also affects the processes establishing physical properties determining quality such as firmness.

The negative ramification of respiration during storage is a loss of food reserves in the stored commodity. While respiration is needed to maintain a level of quality, it always leads to a loss in dry weight of the commodity. In some commodities the loss is minimal because the product is stored for only a short time, for example strawberries (*Fragaria* × *ananassa*), while in others that are often stored for a longer period, the loss can be significant, such as onions (*Allium cepa*) and potatoes (*Solanum tuberosum*). In addition to dry weight loss, quality of some commodities can suffer severely from respiratory use of sugars. A prime example of this is sweet corn (*Zea mays*).

A tremendous amount of heat is released during respiration and this heat must be removed during storage. It is extremely important to know which commodities require cold storage and to ensure that the cooling system selected is capable of removing any excess field heat as well as the heat generated during storage by respiration.

During respiration, the production of 1 mg CO_2/kg/h translates into a refrigeration requirement of 61.2 kcal/t/day or 220 BTU/t/day (Saltveit, 2004a). One BTU (British thermal unit) is the amount of energy it takes to increase the temperature of 0.454 kg of water from 3.8°C to 4.4°C (or the energy needed to raise 1 lb of water 1°F from 39 to 40°F). Refrigeration units are rated for the number of BTUs of heat they are capable of removing from a storage unit per day. This measure is often reported

as tons of refrigeration per day; 1 t of refrigeration is equivalent to the amount of energy required to melt 1 t of ice at 0°C in 24 h.

Where does all the heat come from during respiration? To understand this problem, one needs to examine respiration a little more closely. The main purpose of respiration is to maintain high levels of energy-carrying molecules such as ATP and NADH in cells for cellular maintenance. The major substrate in respiration is usually glucose. The general equation for respiration utilizing glucose as the substrate is:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 686 \text{ kcal/mole}$$

One mole of glucose is equal to 180 g, coming from starch or a simple sugar such as glucose or sucrose. The energy released during the respiration of 1 mole of glucose has three different destinies. A small amount of energy (13 kcal) gets lost in entropy during the breakdown of glucose into simpler components during respiration. Approximately 281 kcal are used to produce ATP while the remaining 392 kcal (57%) is lost as heat. So much energy is lost as heat because at each step of any metabolic reaction some heat is lost. There are hundreds of individual metabolic reactions occurring during the respiration of 1 mole of glucose, thus it is easy to see why so much heat is lost in such a process.

Don't think that respiration during storage is all bad. The synthesis of lycopene in tomatoes during storage, the production of volatiles for aroma and flavor in many fruits as well as the degradation of starch into sugars all depend on the energy produced by respiration.

Ethylene

Ethylene (C_2H_4) is a very important component of postharvest physiology (Saltveit, 2004b). As indicated in Chapter 2, this volume, ethylene is a naturally occurring molecule that is a colorless gas at biological temperatures that easily diffuses through plant tissues. It can exert a biological effect at rates of parts per billion. Ethylene is biosynthesized from the amino acid methionine via *S*-adenosyl-L-methionine (SAM) which is converted into 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase in a highly regulated process. The final step requires ACC oxidase (and oxygen) which converts ACC into ethylene. It is an autocatalytic process meaning that the presence of ethylene above a specific threshold stimulates the production of more ethylene. In vegetative tissues, the level of ethylene is usually below this threshold, thus ethylene production is not stimulated. Similarly in non-climacteric fruits, ethylene remains below the threshold, thus ethylene production remains at a fairly constant level. In climacteric fruits ethylene levels surpass the threshold initiating autocatalytic ethylene production. This is a major problem in storing fruits and vegetables, where in general, inhibition of ethylene production is desired.

Most plant tissues generate small amounts of ethylene to regulate various aspects of growth and development. Since ethylene readily diffuses, it is normally remains at relatively low, biologically active levels in the plant tissues. Any postharvest barriers to such diffusion such as waxes or containers may lead to excessive accumulation of ethylene which could induce autocatalytic production. Ethylene is often produced by wounded tissue, thus any decaying or unsound produce can lead to premature ripening and senescence and spoil the entire lot.

Ethylene is often called the wound hormone or the ripening hormone. While it is produced in response to wounding and it does induce ripening in climacteric fruit and some non-climacteric fruit, it also has other effects (see Chapter 2). Pertinent to this discussion, ethylene stimulates the production of anthocyanins in ripening fruit while it enhances chlorophyll destruction, which is important in degreening many citrus fruit. The positive effects of ethylene on one commodity, for example degreening in citrus, may be highly undesirable in another, for example, degreening of broccoli (*Brassica oleracea* Itlaica Group).

Regulating ethylene during storage

Since ethylene has many diverse effects on different commodities, controlling its levels in storage is crucial in postharvest physiology. Increasing ethylene levels to induce autocatalytic production and the ripening process is easily accomplished using ripening chambers where ethylene gas is introduced into the atmosphere. This is an important practice for point-of-sale ripening of bananas (*Musa* spp.), avocados (*Persea americana*), and tomatoes (*Solanum lycopersicum*).

Keeping ethylene levels low during storage is usually desired to inhibit or prevent ripening. This can be accomplished by: (i) preventing ethylene generation by the product; (ii) removing excess ethylene

from the atmosphere; and (iii) preventing the aberrant introduction of ethylene from external sources, particularly forklifts, into the storage facility.

Maintaining the storage facility at the lowest possible temperature without inducing chilling injury is one way of minimizing ethylene production. Separating ethylene-generating produce from sensitive commodities is another very important consideration in maintaining adequately low levels of ethylene in storage. Storage under controlled or modified atmosphere conditions can also minimize ethylene levels in storage.

Removing ethylene from the storage air with scrubbers or with small sachets inside retail containers can effectively keep ethylene levels acceptable.

One way to control ethylene in storage is to prevent ethylene synthesis using compounds such as aminoethoxyvinylglycine (AVG), aminooxyacetic acid (AOA), and silver salts. Use of these compounds is normally limited to non-food crops. Other sources of ethylene may exist in or near the storage facility, thus prevention of ethylene production by the commodity itself does not necessarily avoid a problem.

Another approach is to prevent the perception of ethylene's presence by treating produce with CO_2 or 1-methylcyclopropene (1-MCP). The inhibitor replaces ethylene at sites of molecular perception within the plant, thus the effect of ethylene is not induced because the ethylene receptor is occupied by the inhibitor rather than ethylene.

Factors Affecting Storage

Many factors affect the storage life of horticultural commodities including: (i) temperature; (ii) respiration rate; (iii) O_2 and CO_2 concentration; (iv) stage of development at harvest; and (v) postharvest pathogens (Fig. 16.1).

Temperature

Respiration and the Q_{10}

Temperature is the most important factor in postharvest physiology because temperature has a profound effect on the rates of biological processes, particularly respiration. Within the normal temperature range of most horticultural crops (0–35°C), respiration increases exponentially with temperature (Saltveit, 2004a). We often describe the sensitivity of a reaction to temperature with the Q_{10} value. The Q_{10} effectively describes the change in the rate of a reaction, in this case respiration, with a 10°C change in temperature. It is derived by dividing the rate of the reaction at the higher temperature by the rate of the reaction at the lower temperature (Saltveit, 2004a). Q_{10} values allow us to estimate respiration rates and therefore storage life at different temperatures, if we know the respiration rate at a specific temperature and Q_{10} values for the commodity. If a commodity has a storage life based on respiration rates of 10 days at 10°C and a Q_{10} value of 2 (for temperatures between 0 and 10°C) and a Q_{10} of 3 (for temperatures between 10 and 20°C), we can determine that it would store for 15 days at 5°C (10 days divided by Q_{10} of 2 = 5 extra days, 10 days + 5 days = 15 days) and 6.7 days at 20°C (10 days divided by Q_{10} of 3 = 3.3 fewer days, 10 days – 3.3 days = 6.7 days).

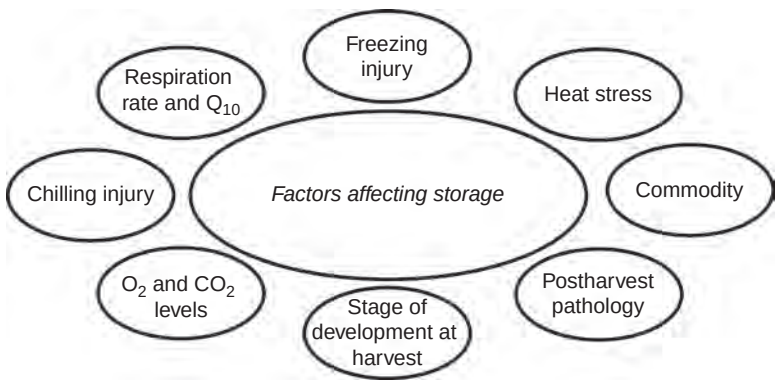


Fig. 16.1. Factors affecting storage of horticultural commodities.

Chilling injury

Temperature is also important in postharvest considerations due to the potential for chilling injury, particularly in tropical and semi-tropical commodities. The temperature at which injury occurs varies among sensitive commodities but is usually somewhere around 10 or 12°C. Some temperate commodities can suffer chilling injury but at temperatures much lower (between 0 and 5°C) (Wang, 2004a). Chilling injury is qualitative damage to the commodity that is often reflected in abnormally high respiration rates during chilling or abnormally high rates once the commodity is returned to non-chilling temperatures. The elevated respiration observed during chilling or soon thereafter is a reflection of the tissues' attempts to remove harmful toxins that accumulate during chilling and to repair damaged membranes and other cellular organelles.

Temperature and duration of exposure are important components of chilling injury. Generally when the temperature is significantly lower than the threshold, injury can occur with a much shorter exposure compared with injury that occurs closer to the threshold temperature. If the exposure is brief enough or at a temperature close to the threshold, the injury may be reversible. However, in most cases the injury becomes non-reversible very quickly. Injury is cumulative in that a number of brief exposures to unsuitable temperatures can add up quickly to extensive injury.

If the exposure to chilling temperatures is significant, a number of symptoms will appear either during chilling or after removal from storage. This is a significant component of chilling injury: the injury may not be observed until after the consumer has purchased the commodity, or the retailer has received a shipment from the wholesaler. Some of the typical symptoms include: (i) surface pitting or lesions; (ii) internal discoloration; (iii) water-soaked tissues; and (iv) failure to ripen properly. Injured commodities are also generally more susceptible to decay particularly by organisms such as *Alternaria* that do not harm healthy tissues (McColloch, 1953). Classic chilling injury symptoms for a number of commodities along with the upper threshold for injury are shown in Table 16.1 (after Wang, 2004a).

Maturity at harvest can greatly influence the extent of injury in crops such as avocado (*P. americana*), honeydew melons (*Cucumis melo*), and tomatoes (*S. lycopersicum*). There are many treatments that have been shown to lessen chilling injury in a number of different commodities. These include: (i) high or low temperature preconditioning; (ii) intermittent warming during storage; (iii) controlled atmosphere or hypobaric storage; (iv) pretreatment before storage with ethylene, abscissic acid (ABA), methyl jasmonate, or calcium; (v) waxing; and (vi) modified atmosphere packaging (MAP).

Freezing injury

Freezing injury occurs when ice crystals form in the commodity and a noted decrease in quality is observed. The freezing point of the commodity does not determine the susceptibility to freezing injury. For example parsnips (*Pastinaca sativa*) and tomatoes (*S. lycopersicum*) both have a freezing point of between –1.1 and –0.6°C yet parsnips can be frozen and thawed several times without any apparent injury while tomatoes that freeze just once are useless. The major symptoms of freezing injury are water-soaked tissue, particularly the skin, and a non-turgid, limp consistency upon thawing.

Commodities are categorized based on their susceptibility to freezing injury (Table 16.2, after Wang, 2004a). Those that are highly susceptible are likely to be injured with even a light freeze. Those that are moderately susceptible might recover from a couple of light freezes. The least susceptible commodities can be frozen and thawed a number of times without any apparent injury.

The intensity of a freezing event is coupled with its duration in determining the extent of injury for many commodities. For example, apples (*Malus domestica*) can take several days of freezing at or just below their freezing point, but would be severely injured by several hours at temperatures just several degrees colder. Another important point about freezing injury is that the organ involved does not necessarily determine the extent of injury, but rather, injury is more related to species susceptibility. For example, leaves of cabbage (*Brassica oleracea* Capitata Group) can withstand many freezes without injury while lettuce leaves (*Lactuca sativa*) are destroyed with one light-to-moderate freeze.

Bruising is a large problem if a frozen commodity is moved while frozen. It is best to let the commodity thaw before inspecting or moving it. Tissues should be thawed at a rate of 4°C/h

Table 16.1. Chilling injury symptoms for different commodities and the upper threshold temperature (°C) causing injury (after Wang, 2004a).

Commodity	Upper threshold temperature (°C)	Injury symptoms
Apples (some cultivars) (*Malus domestica*)	3	Brown core, internal flesh browning and soggy, soft scald
Asparagus (*Asparagus officinalis*)	2	Discolored, limp tips
Atemoya (*Annona* × *atemoya*)	4	Darkened skin, discolored pulp, failure to ripen
Avocado (*Persea americana*)	13	Gray-to-brown flesh discoloration
Banana (*Musa* spp.)	13	Dull skin color upon ripening
Snap beans (*Phaseolus vulgaris*)	7	Russetting and surface pitting
Cucumber (*Cucumis sativus*)	7	Skin pitting, water-soaked tissue
Eggplant (*Solanum melongena*)	7	Skin discoloration, black seeds
Ginger (*Zingiber officinale*)	7	Tissue softening and breakdown
Jicama (*Pachyrhizus erosus*)	18	Surface decay, flesh discoloration
Mango (*Mangifera indica*)	13	Gray skin, uneven ripening
Cantaloupe (*Cucumis melo*)	5	Pitting, skin decay
Honeydew (*Cucumis melo*)	10	Skin discoloration, pitting, failure to ripen
Olive (*Olea europaea*)	7	Internal browning
Orange (*Citrus* × *sinensis*)	3	Pitting, brown rind stain
Papaya (*Carica papaya*)	7	Failure to ripen, off-flavors
Pineapple (*Ananas comosus*)	10	Internal browning, skin stays green when ripe
Potato (*Solanum tuberosum*)	3	Browning, sweet flesh
Tomato (ripe) (*Solanum lycopersicum*)	10	Water soaking
Tomato (mature green)	13	Failure to develop good color when ripening

Table 16.2. Susceptibility to freezing injury of common horticultural commodities (after Wang, 2004a).

Category	Commodity
Highly susceptible	Apricots, asparagus, avocado, bananas, snap beans, most berries, cucumbers, eggplant, lemon, lettuce, limes, okra, peaches, peppers, plums, potatoes, summer squash, sweet potatoes, tomatoes
Moderately susceptible	Apples, broccoli, carrots, cauliflower, celery, cranberries, grapefruit, grapes, storage onions, oranges, parsley, pears, peas, radishes, spinach, winter squash
Slightly susceptible	Beets, Brussels sprouts, cabbage, dates, kale, kohlrabi, parsnips, rutabagas, salsify, turnips

(Lutz, 1936). Faster thawing rates cause significant injury because the membranes are not able to handle the rapid rehydration that occurs with quick thawing. Using slower rates allows more time for ice crystals to damage the cells resulting in greater observed injury once thawed. In nearly all cases, commodities that have suffered any amount of freezing injury have a shorter storage and shelf life than those that have not been frozen at all. In addition, previously frozen products often have lower quality, particularly textural quality and are more susceptible to attack by decay-causing organisms.

Heat stress

Exposure of commodities to excessively high temperatures for a brief period is often used to combat pest pressures, especially fungi. If the temperature is too high for an extended period, metabolism is severely affected. Respiration eventually ceases, enzymes are denatured, and membranes lose their integrity and function. Ultimately tissue death occurs and the commodity becomes useless.

O_2 and CO_2 concentrations

Controlling O_2 concentration in the storage facility is a method for prolonging storage life by reducing respiration of the stored commodity. The main

concern is to ensure that O_2 levels are not too low since this would induce anaerobic respiration which results in off-flavors and foul odors. Most crops will store well at 2–3% O_2, however, it is wise to know the recommended level for each particular commodity. Some crops such as apples can be stored at as low as 1% O_2. The O_2 necessary to maintain aerobic conditions for respiration is coupled with temperature. As storage temperature increases, respiration rates increase and thus so does the O_2 rate required to prevent anaerobiosis.

Increased CO_2 levels are sometimes used to delay senescence and reduce decay in some commodities. High CO_2 levels coupled with low O_2 levels can, however, lead to anaerobic metabolism, thus caution is needed when altering storage gas levels.

Reducing the O_2 or raising the CO_2 concentration may suppress pathogen growth during storage. The reaction of the commodity to altered storage gas concentrations must be considered, as anaerobic respiration in stored produce is undesirable.

Stage of development at harvest

Storage life generally differs among different tissue types and stage of development at harvest. Storage organs generally have a low respiration rate while actively growing tissue such as floral or vegetative buds have a high respiration rate. Maturing tissues such as fruit have an intermediate respiration rate that generally declines as the tissue matures further. The closer to maturity a tissue is at harvest, the lower its respiration is likely to be. Once harvested, respiration slowly declines in non-climacteric tissues such as fruit and storage organs and rapidly declines in actively growing tissues. The rapid decline in actively growing tissues is usually attributed to a depletion of storage reserves. Respiration rates of climacteric fruit after harvest were discussed earlier in this chapter.

Postharvest pathology

Postharvest losses of horticultural commodities often range from 10 to 30% even with the use of modern storage facilities and technologies (Harvey, 1978). Losses can be due to biological or non-biological factors. Non-biological factors contributing to losses include rough handling and improper storage. Biological factors contributing to losses are usually infection by bacteria or fungi as well as infestation by insect pests. Postharvest diseases only occur once pathogens attack the host and cause an irreversible decline in commodity quality.

Fungi normally infect fruit while bacteria normally infect vegetables (Sholberg and Conway, 2004). Infection or infestation may occur at any point along the postharvest chain which can make this a particularly difficult problem to manage (Dennis, 1983).

Weather during production and harvest often has a marked influence on postharvest levels of pathogens by affecting both the field populations of such organisms and the susceptibility of commodities to various pathogens. Warm, wet weather often increases the population of pathogens while at the same time making commodities more susceptible to infection.

Production factors such as cultivar selection, fertility management, irrigation frequency, and pesticide applications also have a huge influence on postharvest losses. Calcium nutrition is very important in managing postharvest losses. Increased resistance to pathogens and prevention of physiological problems are associated with adequate calcium levels in apples (*M. domestica*), potatoes (*S. tuberosum*), and peaches (*Prunus persica*) (Sams, 1994). Nitrogen on the other hand tends to induce greater susceptibility to postharvest losses.

Crop maturity at harvest and postharvest handling are important. Many commodities are harvested slightly immature in order to prolong storage life. As a commodity becomes mature and begins to ripen, it becomes more susceptible to infection by pathogens (Kader, 1985). Some commodities are sprayed with a pre-harvest fungicide to reduce postharvest losses.

Postharvest handling has a major impact on losses during storage. A major concern is sanitation during harvest and packaging. Good sanitation practices should be followed by all workers involved in commodity handling and all equipment used during handling must be cleaned and sanitized regularly. In addition, culls, decayed produce, and trash must be disposed of properly to prevent build up of infectious agents. Another area for consideration to help minimize losses is chemical or biological postharvest treatment. Postharvest treatments must consider the pathogen, its location, time of treatment, commodity maturity, and storage environment. Postharvest fungicide treatment has decreased as organisms become resistant or products lose their label for use. In fact, there are a number of pathogens that

are problematic in postharvest but have no registered fungicides available for their control.

Postharvest biological controls are becoming increasingly desirable as fewer chemicals are available for control and consumer demand for sustainable or organic approaches increases. Many biological control organisms are in the developmental stage and the transfer from the lab to the field is a long process. A major problem with biocontrols is that they don't always provide consistent protection. Some control, however, is better than none at all.

Both UV and gamma irradiation have been considered for postharvest control of storage diseases. There is no evidence that UV radiation reduces decay of packaged fruits or vegetables (Hardenburg *et al*., 1986) and gamma radiation may cause undesirable side effects in some commodities and the equipment needed to provide the radiation is extremely expensive.

Low temperatures are used in commodity storage to reduce the growth rate of pathogenic organisms. The temperature is usually kept as low as possible to retard pathogen growth yet warm enough to prevent chilling injury in sensitive commodities. Exposure to high temperatures, usually by immersing the commodity in hot water, can reduce decay during storage in some commodities such as peppers (*Capsicum annuum*), tomatoes (*S. lycopersicum*), mango (*Mangifera indica*), and papaya (*Carica papaya*) (Spotts, 1984). Reduced humidity can reduce or prevent germination and growth of fungal pathogens. However, reduced humidity during storage can dehydrate a commodity and reduce its quality.

Another important component of postharvest horticulture are issues of food safety, namely, the presence of fecal coliform bacteria (Gould, 1973) and microbial toxins (Hsieh and Gruenwedel, 1990) in stored commodities. Good hygiene by harvest and postharvest workers is the key in preventing fecal coliform contamination. The appropriate management of animals and their waste products near production fields, harvest and processing stations along with the proper composting of manure used in crop production also helps avoid contamination. With some organisms, it is not the organism itself that is harmful to human health, but rather a toxin produced by the organism. Examples include: (i) botulinum toxins produced by the anaerobic bacterium *Clostridium botulinum*; (ii) aflatoxins produced by *Aspergillus* fungi; and (iii) patulin, a toxin produced by *Penicillium* and *Aspergillus* species.

The Commodities

In this section, postharvest issues for a number of horticultural commodities are reviewed (Fig. 16.2). The omission of commodities from this discussion does not reflect any opinion of importance of the commodity by the author. For more information on any listed or not-listed commodity, see Gross *et al*. (2002) or search the Internet.

Culinary herbs

Culinary herbs may be annual or perennial and include: (i) annuals such as basil (*Ocimum basilicum*), chervil (*Anthriscus cerefolium*), coriander or cilantro (*Coriandrum sativum*), dill (*Anethum graveolens*), and summer savory (*Satureja montana*); and (ii) perennials such as chives (*Allium schoenoprasum*), Chinese chives (*Allium tuberosum*), marjoram (*Origanum hortensis*), oregano (*Origanum vulgare*), peppermint (*Mentha piperita*), spearmint (*Mentha spicata*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*; Fig. 16.3), tarragon (*Artemesia dracunculus*), and thyme (*Thymus vulgaris*). Leaves are most often used, however, roots and dried fruits (shizocarps) of cilantro are used as well as chive flowers.

Leaves should be harvested when green and turgid, usually before flowering and stored at 95–100% RH and just above 0°C, with the exception of basil which must not be stored any lower than 12°C or severe chilling injury in the form of blackened foliage will occur. Leaves are often bunched and tied with twine or secured with rubber bands. Packaging in clamshells or polyethylene (poly) bags help reduce dehydration. There are no standardized grades for herbs. Herbs produce little ethylene but are highly susceptible to ethylene injury exhibited as leaf yellowing and abscission (Wright, 2004a, b).

Vegetables and fruits

Root, rhizome, tuber, and bulb vegetables

BEETS Beet (*Beta vulgaris* Crass Group), also known as table beet or red beet, is a biennial producing a fleshy, edible storage root (enlarged hypocotyl). Leaves are also consumed, usually steamed or sautéed and make an excellent substitute for cooked spinach. At the seedling and young plant stage before hypocotyl enlargement, the leaves are often included raw in salad mixes. Root shape, size, color, turgidity, and smoothness

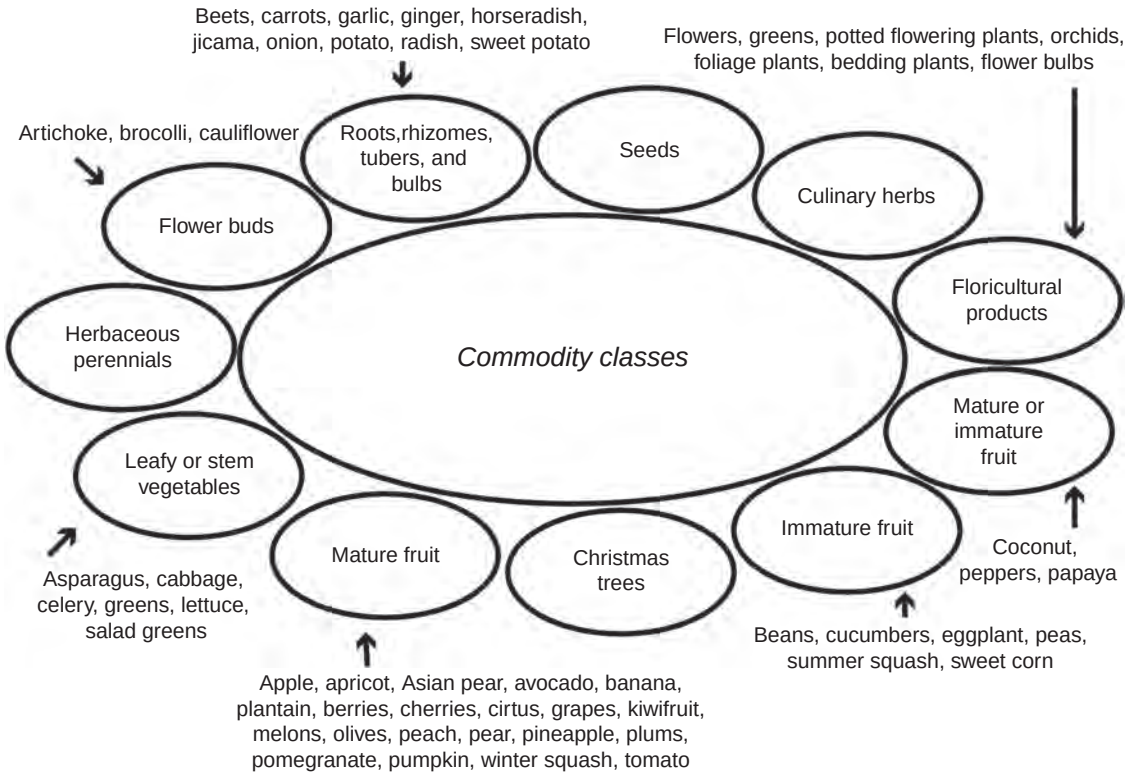


Fig. 16.2. The major horticultural commodity classes.

Fig. 16.3. Sage (*Salvia officinalis*).

(lack of rootlets) are major quality characteristics. The most important quality attribute for beet roots is a lack of zoning, or alternating dark and light concentric rings of flesh. A sharply zoning cultivar 'Chiogga' is marketed as a novelty. Leaves for salad should be small, tender, and free of blemishes.

Roots are harvested 50 days after planting or later but before full maturity is reached, especially if intended for long-term storage. Beets are often sold by the bunch with leaves attached. Beets are pre-cooled to at least 4°C. Bunched beets will keep for 10–14 days at 0°C at >98% RH. Topped beets are stored at 1 or 2°C at 98% RH and retain quality for 8–10 months (Adamicki, 2004a). Beets are not chilling sensitive, thus they should be stored as cold as possible without freezing. Topped beets can also be stored in soil trenches in areas with a sufficiently cold and consistent winter provided the temperature of the pit remains between –1 and 5°C. Beets are not stored under controlled atmospheres. Beets produce little ethylene and are not sensitive to ethylene exposure. The major problem with stored, topped beet roots is decay from gray mold (*Botrytis cinerea*) and black rot (*Phoma betae*).

CARROT Carrot (*Daucus carota*) is a biennial producing a large storage tap root, high in carbohydrates and β-carotene. High quality carrots are solid, straight from shoulder to tip, smooth and

sweet with no bitterness or aftertaste. Carrots are mostly harvested when they are partially mature when the tap root is 1.8 cm or larger in diameter at the shoulder. Carrots used for fresh-cuts are harvested immature to ensure sweetness. Carrots may be bunched or top trimmed. Quick cooling to <5°C is important to maintain a crisp texture and overall freshness. Roots should be stored at 0–1°C and close to 100% RH. Under perfect storage conditions, topped carrots can be held for up to 9 months, however, most carrots are only held for 5–6 months. Bunched carrots can be held for 8–12 days and longevity is enhanced by storing them covered with flaked or shaved ice. Controlled atmosphere storage of carrots does not improve their storability, thus it is rarely used. Carrots are not chilling sensitive. They produce little ethylene, but exposure to exogenous ethylene induces the formation of isocoumarin which imparts a bitter flavor. Bitter flavors also develop when exposed to ethylene, but not in peeled roots. Thus non-peeled carrots should not be stored with other commodities producing ethylene to avoid the development of bitter flavors.

Wilting and rubberiness are indications of storage at low RH which leads to water loss. Rough handling can increase cracking and tip breakage, especially in Nantes-type carrots and other susceptible varieties (Luo *et al*., 2004b). Topped carrots may sprout if storage temperatures are too high. Pre-harvest water stress can lead to the accumulation of terpenoids which results in off-flavors. Surface browning may develop on roots harvested immature. Roots are susceptible to a number of rots during storage including, bacterial soft rot caused by *Pectobacterium carotovora* or *Pseudomonas marginalis*, gray mold (*B. cinerea*), Rhizopus rot (*Rhizopus* spp.), watery soft rot (*Sclerotinia sclerotiorum*), and sour rot (*Geotrichum candidum*) (Snowden, 1992).

GARLIC The main edible portion of garlic (*Allium sativum*) is a bulb consisting of enlarged leaf bases called cloves that are wrapped in dried leaf sheaths attached to a basal stem plate, all surrounded by several layers of dried leaf sheaths. Flower stalks called scapes are also used. Garlic is most often sold fresh, dried and ground, or chopped and preserved in olive oil. Garlic may be harvested at almost any time during its development, but is most often harvested when mature. It is harvested mature when tops have fallen over and dried. High quality bulbs are firm, heavy for their size, have skin color appropriate to the variety and a soluble solids content (SSC) of at least 35% (Cantwell, 2004b). The necks and skins of garlic must be allowed to dry sufficiently (cure) to maximize storage. Garlic can be stored for 1–2 months at 20–30°C at <75% RH. For longer storage up to 9 months, garlic should be stored at –1–0°C at 60–70% RH. Garlic held at 5–18°C loses its dormancy and begins to sprout. Sprouting can be inhibited with a pre-harvest application of the herbicide maleic hydrazide, which is also used to prevent sprouting in onions (*A. cepa*) and potatoes (*S. tuberosum*). Garlic should be isolated during storage to prevent the odor of garlic from being transferred to other commodities. Controlled atmosphere storage at 0–5°C with high (5–15%) CO_2 retards sprouting and decay but may induce a yellow translucent color in come cultivars. Garlic is not chilling sensitive, does not produce much ethylene and is not sensitive to exogenous ethylene.

High field temperatures before harvest may lead to the storage disorder called waxy breakdown. It is characterized by light yellow areas in the clove flesh that darken. Later the clove becomes translucent, sticky and waxy but the skins remain unaffected. The most common rot of garlic in storage is caused by *Penicillium* spp. It is usually only detected after it is in the final stages of development which results in bulbs that are light in weight for their size and individual cloves that are soft and powdery with intact skins.

GINGER Though ginger (*Zingiber officinale*) is a rhizome, a creeping, underground stem, it is often called a root. It is used as a spice, a pickled vegetable, and is used medicinally. It is also dried and ground or preserved in sugar syrup, dried and sold as crystallized ginger. Ginger for pickling and crystallizing should be harvested before it is mature to avoid the fibrous texture that develops at maturity. Immature ginger is bright yellow to brown, with a high sheen and green or yellow non-sprouted buds. Mature ginger is harvested when the shoots wilt and begin to die. Rhizomes have a bright yellow-brown skin that soon loses its sheen and darkens. Rhizomes should be cooled to 12–14°C and can be stored at the same temperature at 85–90% RH for 2–3 months. Problems during storage include wilting, mold growth if condensation occurs on the rhizomes, and rot caused by *Fusarium* and *Pythium* spp. Ginger is chilling sensitive and storage at 12°C or lower leads to loss of skin color, skin pitting,

and internal breakdown (Paull and Chen, 2004b). Ginger does not produce appreciable ethylene and is not sensitive to exposure to exogenous ethylene.

HORSERADISH Horseradish root (*Armoracia rusticana*) is a cruciferous perennial grown for its enlarged tap root which is normally used as a condiment for meat and fish. Long, uniform, firm, smooth roots free from hollow heart with pungent flavor are desired. Roots are harvested after frost has killed the tops, but roots for processing may be harvested earlier. Roots are extremely sensitive to wilting and should be cooled to 4 or 5°C then stored at 0°C at 98% RH, where they will keep for up to 9 months (Adamicki, 2004b). At warmer temperatures, roots loose pungency. Horseradish roots are not sensitive to chilling, produce very little ethylene, and are not sensitive to exogenous ethylene (Adamicki, 2004b). Roots infected by *Verticillium dahlia* in the field exhibit darkened vascular tissues that appear as dark spots in cross-wise sections or dark streaks in longitudinal sections of the root. This discoloration is a major cause of quality loss.

JICAMA Jicama, also called yam bean, (*Pachyrhizus erosus*) is a leguminous root crop that can be eaten raw or cooked. High quality is reflected in large, smooth, firm roots with crisp, succulent white sweetish flesh (Cantwell, 2004c). Most jicama is harvested mature after tops die. Since they are a tropical crop jicama roots are chilling sensitive. Roots exhibit injury, primarily external decay and interior discoloration and loss of crispness, after 1–3 weeks storage at 10°C. Flesh may also appear water soaked or become rubbery. To minimize chilling injury, they should be stored at 12–15°C at 80–90% RH. Under these conditions they will last 2–4 months before they begin to sprout. Once they sprout, jicama roots loose firmness and juiciness. Jicama roots produce little ethylene and are not sensitive to exogenous ethylene. Decay is the major storage problem with jicama and it can be minimized by avoiding injury to the periderm during harvest and handling. Jicama is often cured to increase periderm toughness by holding them at 20–25°C at 95–100% RH for a week.

ONION Onion (*Allium cepa* Cepa Group) is a biennial grown for its bulb of enlarged leaf bases attached to a short stem plate (Adamicki, 2004c). It is normally harvested after the first year of growth, as the bulb quickly flowers in its second year. Scallions are immature onions used for their bulbs and foliage. High quality bulbs should be firm, free from defects and have bulb size, shape, and skin color appropriate for the cultivar. Harvest maturity depends on their use. Scallions and onions for bunching can be harvested at any size. Onions for storage are harvested when 50–80% of the tops have fallen over. Bunched onions and scallions should be quickly cooled to 0°C and covered with crushed ice if possible. Onions for storage should be cooled to 0°C as quickly as possible to inhibit rooting and sprouting during storage. Slow or gradual cooling will not reduce rooting and sprouting during storage. Bunched onions can be stored for 3–4 weeks at 0°C at 95–98% RH. Mild and sweet onions are stored for up to 3 months at 3–5°C. Pungent, dry onions can be stored for up to 9 months at 0°C and 65–75% RH.

Once harvested, onion bulbs are dormant for 4–6 weeks depending on cultivar. A sprout inhibitor may be applied about 2 weeks prior to harvest if onions are to be stored for a long period. Onions for long-term storage are field dried for 2 weeks followed by room curing and drying at 25–27°C until the necks are tight and totally dry. After curing and drying, bulbs are cooled to 0°C for storage. Onions are often stored with other commodities at 3–5°C, but the length of storage is greatly reduced compared with storage at 0°C. Controlled atmospheres may be used for onion storage, particularly pungent cultivars. Low O_2 reduces respiration and prolongs storage life while elevated CO_2 reduces sprouting and root growth. Onions are not susceptible to chilling injury. Bulbs produce little ethylene and they are not particularly sensitive to exogenous ethylene, however, high levels of ethylene (>1500 μl/l) may induce sprouting. Scales may appear translucent or watery after storage due to freezing injury, delayed cold storage after curing, or late harvesting and prolonged field drying at high temperatures. Outer scales may also turn green if exposed to light. Bulbs are also susceptible to various rots during storage, so good sanitation is important for preventing losses.

POTATO The white potato (*S. tuberosum*) is an annual, cool-season crop, grown for its underground tubers that form on the ends of underground stolons as storage organs. Both skin and flesh colors vary widely among cultivars with brown russet, white, red, pink, yellow, and purple

skins covering white, cream, yellow, purple, red, and striated flesh. Tubers may be long and slender or nearly round (Voss, 2004). High quality potatoes should be: (i) clean of soil or defects; (ii) appropriately sized and colored for the cultivar; and (iii) firm and free of skin greening or sprouts. While vine senescence is often used as a criterion for harvest, resistance to tuber skin abrasion (skinning) is a much better harvest index. Sugar content is often used as an additional harvest index for processing potatoes.

Potatoes destined for long-term storage must first be cured for 1–2 weeks at 20°C and 80–100% RH. Once cured, the temperature is lowered by 1 or 2°C/day until the desired storage temperature is reached. The RH should be maintained at 99% to prevent shriveling and bruising during storage. Tubers used as seed potatoes are stored at 4–5°C to minimize sprouting. The conversion of starch to sugars is a key concern for determining storage temperature. In general, starch-to-sugar conversion is greatest at low, above-freezing temperatures. Each cultivar has a characteristic starch-to-sugar conversion temperature profile, thus the cultivar must be considered along with the culinary use when determining the best storage temperature. Tubers for fresh consumption are stored at 7–10°C, to minimize starch-to-sugar conversion. Tubers for frying are stored at 10–15°C to prevent excessive sugar accumulation since many cultivars used for frying have high starch-to-sugar conversion rates at temperatures below 10°C. Cultivars used for making chips often have a high starch-to-sugar conversion rate at temperatures below 15°C, thus they should be stored between 15 and 20°C. Most cultivars will store for 2–12 months depending on temperature and whether or not they are treated with sprouting inhibitors. Controlled atmosphere storage of potatoes is minimal. Potatoes are chilling sensitive. Internal browning occurs at 1–2°C while unacceptable and non-reversible sugar accumulation occurs at 3–4°C. Potatoes produce little ethylene, except wounded tubers which will produce large amounts of ethylene. Tubers are fairly insensitive to exogenous ethylene, however, very high levels can induce sprouting.

There are a number of physiological maladies that affect potatoes during storage including black spot, blackheart, freezing injury, and greening. Black spot occurs primarily on the stem end of the tuber after a physical impact and appears as a dark, black spot at the point of impact. Blackheart is caused by low O_2 levels during storage or transportation and is induced at temperatures >30°C. High respiration rates at these temperatures leads to a lack of O_2 in the center of the tuber which causes cells to die and turn black. Freezing injury can occur at −1°C in the field or during storage. Affected tissue is easily identified as a sharply delineated water-soaked area which results in tissue deterioration after thawing. Greening occurs in tubers exposed to light. It results from a short exposure to high light levels or a longer exposure (1 or 2 weeks) to low level light and leads to chlorophyll and solanine production. Solanine is a heat-stable, toxic glycoalkaloid that is minimally degraded with cooking. Therefore, greened tubers should be discarded. Potatoes are highly susceptible to a number of rots during storage, thus sanitation to prevent infection is important.

RADISH Radish (*Raphanus sativus*) is a root crop in the family *Cruciferae*. It is quick growing and available in many colors and sizes. They are normally eaten raw, as their flesh is sweet to pungent and succulent. High quality radishes are uniform in size, firm, and crisp. Flesh should not be stringy or pithy. Roots are harvested according to size and larger ones are often pithy. They may be sold bunched or topped (Hassell, 2004).

Roots should be pre-cooled to 0–4.5°C then stored at 0°C and 90–95% RH where they will last up to 4 weeks. Bunched radishes will store for 1 or 2 weeks under these conditions. Winter black radishes will store for up to 4 months. Topping radishes helps prolong storage (Fig. 16.4). Controlled atmosphere storage at 1–2% O_2 and 2–3% CO_2 at 0–5°C slightly prolongs storage. Radishes are not sensitive to chilling injury, produce little ethylene and are not sensitive to exogenous ethylene.

SWEET POTATO The sweet potato (*Ipomea batatas*) is a perennial fleshy storage root often grown as an annual. Sweet potatoes come in a variety of colors including white, creamy white, yellow, orange, red, and purple. They may be sweet to bland and mild to strongly flavored with flesh that can range from firm to very soft. Harvest is based solely on root size as there is no specific developmental stage defining maturity: roots will continue to enlarge until they die of rot or anaerobiosis or the tops are killed by frost. Harvested roots must be cured by storing them at 29°C at 90–97% RH for 4–7 days. Storage rooms must be vented during curing. Curing helps

Fig. 16.4. Topped (left) and bunched (right) radishes (*Raphanus sativus*).

heal wounds caused in harvest and handling and also helps develop enzymes responsible for flavor development during cooking (Kays, 1997). As roots cure, the outer layer of parenchyma cells dehydrates while the underlying cells become suberized. A lignin-like wound periderm then develops underneath the suberized layer, and curing is complete when this layer is three to seven cells thick. After curing, roots are stored for up to a year at 14°C and 90% RH. Roots are not typically stored under controlled atmospheres. Roots are chilling sensitive at temperatures below 12°C. Symptoms include: (i) root shrivelling; (ii) surface pitting; (iii) internal browning due to the formation of chlorogenic acid and other phenols (Walter and Purcell, 1980); and (iv) hardcore formation. Hardcore formation does not become apparent until roots are cooked. Roots are sensitive to high levels of ethylene but only to levels higher than those encountered in normally ventilated storage rooms. If roots are exposed to anaerobic conditions prior to harvest (heavy rains with poor drainage), roots often decompose rapidly once in storage, emitting a distinct fermented, sour smell. Roots may also become pithy during storage. Many organisms can cause root rot during storage. Internal corking, characterized by internal necrotic lesions, is caused by a virus infecting most cultivars and may develop during storage. Sweet potato weevils (*Cylas formicarius*), fruit flies (*Drosophila* spp.), and soldier flies (*Hermetia illucens*) are serious storage pests. The sweet potato weevil enters storage on infected roots, thus roots should be examined for their presence before storage and roots discarded if weevils are present. Fruit and soldier flies often contaminate rotten or injured roots. Good sanitation during processing and storage are important for controlling many of the postharvest pests.

Leafy/stem vegetables

ASPARAGUS Asparagus (*Asparagus officinalis*) is a perennial in the family *Liliaceae* prized for its tender shoot. When harvested either white (blanched) or green, the shoot consists of a thick stem with only scale leaves at each internode. High quality asparagus spears are harvested when 10–15 cm in length and should be firm, straight, tender, and glossy with tightly closed tips. Green spears should be green their entire length as white butt ends are not desirable even though they tend to resist decay during handling as compared with spears with green butts. Asparagus continues to elongate after harvest and is one of the most perishable horticultural commodities (Luo *et al*., 2004a) and should be cooled to 0°C as soon after harvest as possible. Spears become tough and loose flavor if they are not cooled as quickly as possible. They can be stored at 0°C and 95–99% RH for 14–21 days. Spears should be stored vertically to prevent a geotropic tip response to horizontal storage. As spears senesce, buds underlying the bracts on spears begin to grow causing the problem known as feathering. Proper storage length at the correct temperature helps reduce its appearance. Controlled atmosphere storage at CO_2 levels of 5–10% helps to slow toughening and prevent decay. Storage at O_2 levels less than 2% leads to the development of off-odors and tip discoloration. Spears are sensitive to chilling and may be injured after 10 days at 0°C. Symptoms of injury include a loss of sheen and glossiness of the entire spear, a general limp appearance as well as graying of the tips. Severe injury may lead to darkened spots or streaks near the tips. Spears produce little ethylene, however, exposure to exogenous ethylene hastens toughening.

CABBAGE Cabbage is grown for its terminal vegetative bud which may be smooth, red or green and round or pointed (*Brassica oleracea* Capitata Group), or round, green and savoyed (Prange, 2004a). Cabbage may be eaten raw, cooked or fermented. Heads should be firm and brightly colored at harvest. Maturity is based on size and head firmness or density. The head should feel heavy for its size, a sign of a nice, dense head. Immature heads

are prone to decay while over-mature heads are likely to split. Cabbage should be cooled and will store at 0°C at 98–100% RH for up to 6 months. Careful temperature control is necessary as cabbage will freeze at −1°C and will senesce quickly at 1°C. Cabbage is often stored under a controlled atmosphere of 1.5–5% O_2 with 0–8% CO_2, depending on cultivar, type, and maturity. Cabbage is not sensitive to chilling injury and produces very little ethylene. It is sensitive to exogenous ethylene which can accelerate senescence.

CELERY Celery (*Apium graveloens*) is grown primarily for its succulent, thick, green-to-white petioles. The leaves are also edible and tend to be more strongly flavored than the stalk. High quality celery should have straight, well-formed, light green stalks. It is harvested based on size, usually when most of the plants in the field have reached 35–41 cm in height and outer petioles have not become pithy. Celery should be cooled quickly then stored at 0°C with >95% RH for up to 7 weeks (Luo *et al*., 2004c). Interior petioles will continue to grow if the temperature is >0°C. Celery is not sensitive to chilling and produces little ethylene. Exposure to exogenous ethylene may cause accelerated senescence if it occurs at temperatures above 10°C. The major postharvest problem associated with celery is pithiness. Pithiness occurs when parenchyma cells in the petiole break down to form aerenchyma tissues. Pithiness is induced prior to harvest by cold stress, water stress, induction of bolting or root infection but is delayed with proper storage temperature. Blackheart is a disorder caused by calcium deficiency and water stress which causes death of cells in internal leaves which leads to brown discoloring which soon turns black. Boron deficiency leads to cracking of the petiole on the interior side.

GREENS FOR COOKING A number of vegetables are grown for their leaves which are mostly eaten cooked rather than raw. These include collards and kale (*Brassica oleraceae* Acephala Group), spinach (*Spinacia oleraceae*), rape (*Brassica napus*), mustard (*Brassica juncea*), and turnips (*Brassica rapa*) (Rushing, 2004b). Spinach may be harvested very young for use in salads but is most often harvested mid-maturity for cooking. The other greens are harvested as full-size, mature leaves that have not begun to senesce. Leaves should be uniform and free of defects, decay, and insects. Leaves should be cooled as quickly as possible after harvesting and stored at 0°C and 95–98% RH for about 2 weeks. Leafy greens are not sensitive to chilling. Leaves produce little ethylene but are very sensitive to exogenous ethylene which causes rapid yellowing and senescence.

LETTUCE There are four main types of lettuce (*Lactuca sativa*) grown for leaves used primarily in salads, soups, or as a garnish. The four types are: (i) iceberg (crisphead); (ii) butterhead (bibb, Boston); (iii) cos (romaine); and (iv) leaf (Saltveit, 2004d). Crisphead lettuce forms heavy heads of crisp, brittle leaves with prominent veins. Butterhead cultivars form open heads of softly textured, tender leaves. Cos lettuce does not form a head, but rather consists of tightly packed upright crisp leaves. Leaf lettuce does not form a head, but rather produces large, softly textured leaves with an open and spreading habit. All lettuce is extremely fragile and should be handled gently at all times. Leaves are harvested at many stages. Lettuce should be pre-cooled then stored at 0°C at 98–100% RH where it will last for up to 4 weeks. Lettuce will be freeze damaged at −0.2°C, thus extreme care must be taken when regulating the storage temperature. Lettuce produces little ethylene but senesces very rapidly if exposed to exogenous ethylene.

Controlled atmosphere storage at 1–3% O_2 and <2% CO_2 reduces some postharvest problems such as russet spot, pink rib, and brown stain. Russet spot is caused by oxidation of phenolic compounds produced in response to exposure to ethylene. It appears as brown oval spots on midribs which occasionally spread to blade tissues in severe cases. Brown stain is caused by exposure to >2.5% CO_2 and appears as large brown stains on leaf midribs. Pink rib is a pink discoloration of the midrib whose cause is unknown.

SALAD GREENS Many other leafy greens are used in salads besides lettuce (Fig. 16.5), including *Valerianella locusta, Valerianella olitoria* (corn salad, lamb's lettuce, field salad, mâche), *Taraxacum officinale* (dandelion), *Rumex scutatus* (sorrel), *Montia perfoliata* (*Claytonia perfoliata*) (claytonia), *Brassica rapa* (mizuna), and *Eruca vesicaria* (arugula). Leaves are usually harvested young and immature for best quality. Salad greens should be cooled to 0–2°C at 95–100% RH where they will store for 7–14 days. Salad greens are not chilling sensitive and they produce little ethylene. Exposure to exogenous ethylene will lead to premature

Fig. 16.5. Mixed baby salad greens.

yellowing and senescence. Leaves are extremely delicate thus must be handled gently throughout the entire production and marketing chain.

Edible flower buds

ARTICHOKE The globe artichoke (*Cynara scolymus*) is a perennial grown for its immature flower bud that resides on a fleshy central base, all enclosed by a cone of short, thick bracts (Wang, 2004b). Good artichokes will have turgid, tightly closed bracts and feel dense and heavy for their size. Buds are harvested when they reach a size appropriate for the cultivar and the intended market. Buds should be cooled to below 5°C as quickly as possible then stored at 0°C and >95% RH for up to 2 weeks. Controlled atmosphere offers no benefit compared with storage at 0°C. Artichokes are not sensitive to chilling or ethylene and produce very little ethylene. The major postharvest problems with artichoke are cosmetic issues resulting from rough handling.

BROCCOLI Broccoli (*Brassica oleracea* Italica Group) is grown for its immature inflorescence. High quality broccoli should be firm, bright green, tight with no signs of bolting (flowering) or senescence. Broccoli is stored at 0–2°C with 98–100% RH for up to 3 weeks. Icing is often used to maintain temperature and humidity around the heads, as broccoli generates considerable heat due to a high respiration rate. Storing in a controlled atmosphere of 1–2% O_2 with 5–10% CO_2 at 0–5°C can double storage life (Toivonen and Forney, 2004). Broccoli is not chilling sensitive. Broccoli generates very little ethylene but is sensitive to exogenous ethylene which causes severe floret yellowing.

CAULIFLOWER Cauliflower (*Brassica oleracea* Botrytis Group) is grown for its head of condensed and malformed flower buds known as curds. Heads should be white, firm, and compact when harvested, with no protrusion of flower parts (also known as riciness). Heads should be cooled and stored at 0°C at 95–98% RH where they will last up to 3 weeks (Forney and Toivonen, 2004). Heads should not be iced, as free-standing water on curds is not desirable and may induce decay. Cauliflower is not chilling sensitive and does not produce much ethylene. It is very sensitive to exogenous ethylene which causes curd discoloration. Boron deficiency in cauliflower may cause brownish discoloration of the curd and pith and may also cause hollow stems. Curds may also taste bitter if deficient in boron. Riciness is induced by seedling exposure to temperatures >20°C before curd initiation, or to 7°C after curd formation. Thus warm temperatures must be avoided before curd initiation and cool temperatures must be avoided after curd formation.

Immature fruits

BEANS Snap beans (yellow wax beans and green beans) (*Phaseolus vulgaris*), runner or flat beans (*Phaseoulus coccineus*), and long beans (*Vigna sesquipedalis*) are all legumes grown for their immature fruit (Cantwell, 2004a). High quality fruit should be firm, tender, without a tough vascular string and be the size and color appropriate for each type. Seeds should be tender as indicated by little or slight bulging of the pod. Beans are harvested 8–10 days after flowering, cooled rapidly to 5–7.5°C and stored at the same temperature with 95–100% RH for 8–12 days. Storage at temperatures below 5°C leads to chilling injury and some cultivars exhibit chilling injury symptoms even at the recommended storage temperature; storage at

higher temperatures leads to unacceptably rapid quality loss observed as dehydration, yellowing of green-fruited cultivars, and excessive seed growth. Chilling injury symptoms include: (i) a general opaque coloring of the fruit; (ii) surface pitting; and (iii) the development of discrete brown spots on the surface which quickly become susceptible to decay. Controlled atmosphere storage at 2–5% O_2 and 3–10% CO_2 can reduce discoloration and decay. Beans produce little ethylene and exposure to exogenous ethylene hastens color loss, causes browning, and reduces storage life by up to 50%.

CUCUMBER Cucumbers (*Cucumis sativus*) are grown for their immature fruit called pepos, which are modified berries usually consisting of three united carpels (Saltveit, 2004c). Fruit are produced on indeterminate vines, although some determinate cultivars are available (the so called 'bush' form). Fruit are round or oblong and cylindrical with small warts with spines (originating as trichomes) on the skin or rind. High quality fruit are harvested immature with crisp white flesh and small or no seeds (parthenocarpic cultivars). Cucumbers are non-climacteric even though a spike in ethylene production is observed just prior to a rapid loss of chlorophyll as fruit mature and ripen. Immature fruit are highly sensitive to ethylene exposure. Fruit exposed to exogenous ethylene rapidly lose chlorophyll and become extremely susceptible to decay. Cucumbers are sensitive to chilling at temperatures less than 10°C, thus they are stored at 10–12.5°C at 95% RH for 1–2 weeks. Chilling injury is observed as surface pitting, the development of water-soaked lesions in the flesh, and loss of chlorophyll from the rind. Bruising is common in poorly handled fruit. Controlled atmosphere storage is rarely used for cucumbers.

EGGPLANT The eggplant (*Solanum melongena*) is an annual solanaceous (nightshade) crop grown for its immature fruit. Fruit may be oval, round, pear shaped or long and slender and may have skin that is white, purple, yellow, lavender, or striped. Cultivars are normally grouped into American, Japanese, Italian, Philippine, Thai, or Chinese types. High quality fruit should have a nice green calyx, firm flesh and shiny skin, and a shape and size appropriate for its type. Fruit are harvested at various stages, but become pithy and bitter with hardened seeds and tough skin as they mature. Fruit are particularly sensitive to compression bruising and should be handled gently and stored in single layers. Fruit should be cooled to 10°C as quickly as possible and stored at 10–12°C with 90–95% RH for up to 2 weeks. Fruit quality deteriorates rapidly in storage, thus they should be consumed as soon as possible after harvest. Controlled atmospheres are not used for eggplant storage. Fruit can be wrapped in plastic film to create a modified atmosphere which helps reduce weight loss and quality deterioration due to water loss (Siller-Cepeda, 2004). Eggplants readily absorb odor, especially ginger, garlic and onion, thus they must be stored separately. Surface pitting and brown surface scalding are symptoms of chilling injury caused by fruit exposure to temperatures less than 10°C. Seed and flesh browning soon follow. Eggplant have a low rate of ethylene production (0.1–0.7 μl/kg/h at 12.5°C and are moderately sensitive to exogenous ethylene >1 μl/l. Calyx abscission and tissue browning may result from exposure to high levels of ethylene.

PEA Peas (*Pisum sativum*) come in three edible types: (i) the garden or green pea where only the seeds are consumed; (ii) snow peas where undeveloped seeds and the pod are consumed; and (iii) snap peas where well-developed seeds and the thick-walled pod are consumed. All three types are produced during cool weather, usually the spring. High quality peas should be harvested at a type-appropriate stage and cooled to 0°C for storage at 95–98% RH for 1–2 weeks. Peas are not chilling sensitive but they are sensitive to exogenous ethylene even though they produce little ethylene themselves (Morris and Jobling, 2004). Exposure to ethylene results in pod yellowing and increased decay.

SUMMER SQUASH Summer squash (*Cucurbita pepo*) fruit are pepos, that are harvested immature for up to 1 week after anthesis to ensure that they are tender, firm, shiny, and flavorful (McCollum, 2004). Of the six horticultural groups of summer squash (cocozelle, crookneck, scallop, straightneck, vegetable marrow, and zucchini), zucchini is the most widely grown. High quality fruit should be young, firm, shiny, and free of defects. Dull fruits indicate senescence and overdevelopment. Fruit must be handled carefully to prevent injury to the skin, which greatly reduces their value. Squash should be cooled to 5–10°C at 95% RH where they will store for no longer than 2 weeks. Summer squash

are chilling sensitive and should not be exposed to temperatures less than 5°C. Symptoms of chilling injury include surface pitting, and accelerated decay and water loss once they are transferred to non-chilling temperatures. Injury is usually not observed at chilling temperatures. Fruit produce low amounts of ethylene and yellowing of green-fruited cultivars may occur if exposed to exogenous ethylene.

SWEET CORN Sweet corn (*Zea mays*) is a member of the grass family producing ears of highly perishable kernels (Fig. 16.6). Ears are perishable in the sense that their quality declines very quickly after harvest due to changes in carbohydrate status of the kernels. The traditional sweet corn cultivars are known as 'sugary 1' (su1), containing about twice the sucrose of field corn and eight to ten times the amount of water-soluble polysaccharide, which imparts a creamy consistency to the kernels (Brecht, 2004b). More recently developed cultivars are known as 'shrunken 2' (sh2) which have twice the sugar as field corn but no water-soluble polysaccharide. The *sh2* gene inhibits starch formation which doubles the shelf life of cultivars carrying it. A third type is known as the 'sugary-enhancer' (su1/se), where the *se* gene modifies the *su1* gene so that the water-soluble polysaccharide content is not lost. These newer cultivars are collectively referred to as 'supersweet' corn.

The highest quality sweet corn has uniform size and color (white, yellow or bicolor) kernels that are sweet, plump and tender, fully filled ears covered by a tight, green husk, and all free of injury, insects, and decay. All sweet corn loses flavor during storage. The taste of su1 and su1/se cultivars becomes starchy while sh2 cultivars become watery and bland. Ears are harvested when the ears are plump and full and the silks have just turned brown and are drying. Kernels of su1 and su1/se cultivars should have a milky endosperm at maturity. The sh2 cultivars always have a watery endosperm, thus kernel consistency is not a good indicator of maturity in these cultivars.

Sweet corn must be cooled quickly after harvest to 0°C. It should be cooled in small batches, and stored in small wooden crates or baskets to minimize heat build up due to the high respiration rate during storage. Top icing crates or baskets is often used to: (i) maximize cooling during storage; (ii) remove heat of respiration; and (iii) keep the husks fresh and hydrated. In general, sweet corn can only be held in storage for a few days before quality rapidly deteriorates. Under the best conditions, sh2 cultivars may be stored for up to 2 weeks. Sweet corn is not sensitive to chilling, produces little ethylene,

Fig. 16.6. Sweet corn (*Zea mays*) nearly ready to harvest.

and is not sensitive to exogenous ethylene. The major postharvest problem with sweet corn is loss of kernel quality.

Mature fruits

APPLE The domesticated apple (*Malus domestica*) is a perennial in the family *Rosaceae* believed to have originated in south-eastern Europe. The skin of the apple contains many lenticels as well as cuticular cracks which are important in gas exchange during storage. Stage at harvest greatly affects longevity in storage. Immature fruit will have poor quality and are prone to storage disorders such as bitter pit and superficial scald. Overly mature fruit will lack firmness and flavor quality will diminish rapidly during storage. Fruit that are just about mature are usually harvested to prolong storage while maintaining the highest quality possible.

Quality is extremely important as most consumers want a blemish-free, sweet, crisp apple. Fruit size and skin color (both under color and over color) are paramount in grading. Any blemishes or defects in appearance or texture are unacceptable, even though some defects are often accepted in the organic market. Minimal flesh firmness, acidity level, and SSC vary with cultivar.

Apples are climacteric and generate varying amounts of ethylene depending on cultivar and stage of maturity. Elevated levels of ethylene are associated with ripening. Early-season cultivars generally have high ethylene production rates, ripen quickly, and have a short storage life while later-season cultivars produce little ethylene until the climacteric, ripen slowly, and have a longer storage life. Monitoring and adjustment of storage ethylene levels is crucial in postharvest apple management. This is accomplished most often via low temperature storage and controlled atmosphere storage. The compound 1-MCP which is structurally related to ethylene is applied at low levels to interfere with ethylene action by preventing the attachment of ethylene molecules to receptors in plant tissues. Best results are obtained when fruit is treated soon after harvest and before it has progressed along the maturity/ripening chain. Besides delaying ripening while maintaining SSC and firmness during storage, 1-MCP prevents scald, a serious postharvest disorder of apples characterized by irregular, patchy, brown or gray skin discoloration.

Most apples are stored in 1 bushel boxes; 1 bushel of apples weighs approximately 18.2 kg. The number of apples per bushel is usually indicated on the box. Most apples are stored in controlled atmospheres at 1–4°C at 90–95% RH depending on the cultivar. Postharvest physiological maladies afflicting apple are separated into three classes: (i) those that develop only while fruit are on the tree; (ii) those that develop on the tree or in storage; and (iii) those that develop only in storage.

Watercore is a physiological condition that develops while the fruit is still on the tree. Intercellular air spaces within the fruit flesh become filled with sorbitol and appear water soaked. It develops on the tree as the fruit matures and nights are cool. In 'Delicious' strains, watercore is a serious problem as it leads to breakdown during storage while watercore in 'Fuji' strains is desirable for the sweetness it imparts to the fruit.

Bitter pit is an example of a malady that develops on the tree or in storage. It is characterized by the development of pits in the flesh either near the surface or deep within the fruit, caused by low levels of calcium in the fruit. The pits eventually turn brown. Cultivars vary in their susceptibility to bitter pit, and in those that are susceptible, the more immature the fruit is at harvest, the more likely it is to suffer from bitter pit. Excessive pruning, high temperature, or drought often exacerbates the problem of bitter pit. Postharvest treatment with calcium may reduce the development of bitter pit in storage.

The disorders that only develop during storage include senescent breakdown, chilling injury, and injury caused by an inappropriate storage atmosphere. Senescent breakdown develops in fruit that are harvested at an overly mature stage. It is characterized by a general loss of flavor quality and tissue softening.

Storage disorders associated with temperature include low temperature breakdown, brown core, and internal browning. Susceptibility depends on cultivar and problems are exacerbated with a cold, wet growing season. Low temperature breakdown leads to brown discoloration of vascular tissues and the flesh with the maintenance of a ring of unaffected tissue just below the skin. Brown core, also known as coreflush, begins with browning of flesh near the core, which proceeds to affect the entire fruit. Internal browning is only observed once the fruit is cut and appears as graying of the flesh. Both coreflush and internal browning are often associated with high levels of CO_2 in storage.

Superficial scald appears as an irregular discoloration on the skin after long-term storage. It can be reduced with a postharvest treatment of fruit with diphenylamine (DPA), however, DPA is not universally allowed as a postharvest treatment. Scald can be reduced with low O_2 and low ethylene storage.

Soft scald appears as irregular, sharply defined areas of soft, light brown tissue on the skin that may penetrate the flesh. Harvest maturity and storage temperature must be closely regulated for susceptible cultivars.

If O_2 levels are too low during storage, a loss of flavor and an increase in fermentation odors develop over time. Additionally, a purplish brown color may develop on the skin of red cultivars and brown soft patches resembling soft scald may occur. Fruit may also soften unexpectedly. Excess CO_2 may lead to wrinkled, depressed patches of skin on the greener side of fruit. Internal symptoms may include tissue browning or cavity formation in the flesh (Watkins *et al*., 2004).

APRICOT The apricot (*Prunus armeniaca*), native to China, is highly prized for its sweet, flavorful fruit that can be eaten fresh or dried. Once harvested, fruit softens quickly and is susceptible to bruising and has a short shelf life. In general, the highest quality apricots have an SSC of >10%, titratable acidity (TA) of 0.7–1.0%, and flesh firmness of 8.9–13.3 N/cm^2.

Fruit are hand-harvested firm mature when the under color changes from green to yellowish, the exact nuances of harvest color varying with cultivar and shipping distance. Depending on market destination, fruit may be bulk packed in 10 kg containers or tray packed in single- or double-layer containers. Fruit are stored for 1 or 2 weeks at –0.5–0°C at 90–95% RH. Fruit may be shipped in a controlled atmosphere of 2–3% O_2 and 2–3% CO_2. Low O_2 (<1%) may lead to off-flavors and high CO_2 (>5% for >2 weeks) may lead to flesh browning and loss of flavor. Ethylene induces ripening in apricot and may also encourage fungal growth on fruit.

Apricots are susceptible to chilling injury if stored at temperatures >0 and <5°C, which appears as loss of flavor, flesh browning or gel breakdown. Gel breakdown develops at temperatures between 2.2 and 7.2°C when fruit are stored for a long time. Flesh begins to look water soaked and later may become spongy or gel-like and finally brown. Pit burn develops in fruit exposed to temperatures above 38°C before harvest. Flesh around the pit softens and turns brown (Crisosto and Kader, 2004e).

ASIAN PEAR Asian pears (also called Chinese pears, Japanese pears, Oriental pears, sand apples, salad pears, and apple pears) are a group of pome fruits derived primarily from *Pyrus ussuriensis* and *Pyrus serotina* with a flavor reminiscent of a European pear and the texture of a crisp apple, even when ripe.

Asian pears are very sensitive to bruising and skin abrasions and are often wrapped in foam nets and boxed in single layers to prevent injury during storage and transit. Fruit remain quite firm even when ripe, are juicy and high in SSC. Fruit are harvested primarily based on a change in ground color from green to yellow or brown, depending on cultivar. Fruit should not be pre-cooled before storage as this tends to induce a malady called fresh spot decay especially in large or overly mature fruit. Fruit are stored at 0°C at >90% RH. Fruit are quite sensitive to ethylene, thus ethylene must be controlled in storage.

Fresh spot decay is internal and appears as spots of brown tissue or cavities in the flesh, particularly near vascular tissues towards the stem end of the fruit. The disorder may appear while the fruit is still on the tree, but becomes more pronounced after 2–6 weeks of storage. The cause remains unknown.

Another internal postharvest problem with Asian pears is internal browning and core breakdown where brown, water-soaked areas in the core or flesh develop during storage. Fruit that remain on the tree for more than 180 days are most likely to develop this problem, thus fruit are usually harvested when nearly all the fruit on a tree are still green.

Low O_2 in storage (level varies with cultivar) leads to discolored surface skin depressions while high CO_2 levels (>5%) lead to core or flesh browning. Fruit are also susceptible to watercore and scalding, much like that described for apples (Crisosto, 2004).

AVOCADO Avocados (*Persea americana*), native to Central America and Southern Mexico, are classified into three races: (i) West Indian; (ii) Mexican; and (iii) Guatemalan. West Indian avocados are tropical with large fruit and relatively low oil content. The Mexican race is subtropical with smaller, thin-skinned fruit of higher oil content.

Guatemalan avocados are subtropical with round, thin-skinned fruit with intermediate oil content. Many commercial cultivars are hybrids of these three races (Bergh and Lahav, 1996).

High quality avocados at harvest should be of the appropriate size for its race, and have good skin color free from skin defects including spray residues. Ripe fruit should be free of postharvest defects including bruising and flesh graying. Avocados have significant oil content, sometimes as high as 30% by fresh weight. The oil quality is similar to olive oil, being approximately 75% monounsaturated, 15% saturated, and 10% polyunsaturated omega 6, and is considered a type of healthy oil.

The percentage dry matter of an avocado is closely related to its oil content, and is therefore widely used as a maturity index. Optimum percentage dry matter at harvest varies with cultivar and ranges from 17 to 25%. Fruit are harvested mature, but not ripe. Fruit can hang on the tree for months as they will not ripen until removed from the tree. The longer fruit remains on the tree, the faster it ripens once harvested. Once harvested, fruit are rapidly cooled to delay ripening to store at a temperature of 5–12°C, depending on cultivar, at 85–95% RH.

Controlled atmosphere storage is often employed, particularly when shipping fruit long distances. The atmosphere used varies with cultivar but is generally 2–5% O_2 and 3–10% CO_2. These levels reduce ethylene generation, respiration, ripening, and softening. Low O_2 injury may occur if O_2 levels fall below 2% and results in irregular brown patches on the skin and flesh browning as well. Elevated CO_2 levels (>10%) can lead to skin discoloration and the development of off-flavors. The use of 1-MCP for delaying ripening is still in its experimental stage. Step-down storage temperatures are often used when storing avocados by decreasing the storage temperature by a degree or two each week until the fruit reach a minimum of 4°C. The storage starting temperature varies with cultivar.

The main postharvest problem limiting long-term storage of avocados is chilling injury, internal and external, which are caused by very different storage conditions. Internal injury appears as grayish brown flesh near the base of the fruit and particularly around the seed. Vascular browning also appears; it begins at the base of the fruit and progresses towards the stem end as the problem develops. Softening of the flesh also occurs. Internal injury often begins to appear after 4 or more weeks in storage at 6°C and is exacerbated by ethylene exposure.

External chilling injury appears as irregular black patches on the skin and occurs after fruit exposure to temperatures <3°C. It often becomes more pronounced after removal from storage. Fruit that are more mature are less susceptible to external chilling injury.

Mature avocados produce little ethylene, however, once fruit begins to ripen ethylene production accelerates dramatically. Ethylene from ripening fruit can initiate ethylene production in mature, non-ripe fruit which are extremely sensitive to ethylene. Many retail outlets expose avocados to ethylene before displaying them for sale to induce rapid ripening (Woolf *et al*., 2004).

BANANA AND PLANTAIN Bananas and plantains are derived from *Musa acuminata* and *Musa bavisiana* and are fruits eaten for their fleshy pulp which is covered by the ovary wall or peel (Kerbel, 2004). The pulp is derived from the innermost layers of the ovary wall and is consumed either raw or cooked. Plantain pulp is much starchier than that of bananas and is usually consumed cooked, even when ripe. Both plantains and bananas are seedless. Both must be harvested mature, but not ripe, as pulp texture is often unacceptable if harvested ripe. Fruit are ripened after shipping either under controlled conditions or naturally. The main harvest index used for both is the time from the emergence of the bunch from the pseudostem and the individual fruit length and diameter. Bananas are shipped and stored at 13–14°C while plantains are shipped and stored at 9–12°C, both at 90–95% RH. Controlled atmosphere storage is often used during shipment of both bananas and plantains and MAP using poly bags is beneficial for prolonging storage life and delaying ripening and senescence. Neither fruit are pre-cooled, as both are susceptible to chilling injury at temperatures below 13°C which is observed as brown or black streaks on the peel or as a grayish color on ripe fruit. In severe cases, the flesh may turn brown or black and may develop an off-flavor. Both fruits are sensitive to exogenous ethylene depending on fruit maturity, temperature, ethylene concentration and length of exposure. Exogenous ethylene even at very low levels will induce ripening. Controlled ripening is nearly always used with bananas while plantains may be ripened under controlled conditions

or allowed to ripen naturally. Fruit are ripened by exposing them to 10–1000 µl/l ethylene in sealed chambers for 24–48 h at 14–18°C. The concentration used varies with ripening facility, but the higher concentrations are normally used to ensure uniform ripening. Rooms must be vented immediately after ethylene treatment to prevent CO_2 build up which can inhibit ethylene-induced ripening. Peel color on a scale from 1 to 7 is used as an indicator of ripeness, with 1 indicating dark green, 6 indicating fully yellow, and 7 indicating brown flecking of yellowed fruit. Fruit are usually marketed at stage 3–4.

A disorder called 'maturity bronzing/maturity stain' sometimes develops 20–30 days before harvest if the crop experiences water deficits along with hot, humid weather during bunch emergence. Eating quality is not affected since the disorder is primarily a discoloration of the skin. Stained fruit are not marketable. If fruit are exposed to temperatures above 30°C, ripening can be irreversibly inhibited.

BLACKBERRY Blackberries (*Rubus* spp.) are grown for their soft, sweet, juicy succulent fruits in many areas of the world. While their root systems are perennial, their fruit-bearing shoots are either annual (primocane fruiting cultivars) or biennial (floricane fruiting cultivars). Blackberry-raspberry hybrids include tayberry, loganberry, youngberry, and boysenberry. Fruits are composed of numerous drupelets attached to a common receptacle which remains as a harvested part of the fruit. High quality fruit are fully black in color, regularly shaped, firm, and free of sunscald. Sunscald can appear as individually whitish-pink discolored drupelets on an otherwise normally colored fruit. Fruit should be harvested as ripe as possible to maximize blackberry flavor and sweetness. The best maturity indices are fruit color and fruit-removal force. Fruit should be harvested when they are a dull black color (as opposed to shiny) and are able to be released from the plant with gentle force. Shiny, non-ripe fruit are often extremely acidic and unpalatable. Fruit are fragile, thus they should be harvested into containers from which they will be sold (generally 1 or 0.5 pint containers, equivalent to 473 ml and 237 ml, respectively) and cooled to <5°C as quickly as possible. Fruit can be stored for 2 days to 2 weeks, depending on ripeness at harvest, at –0.5–0°C at >90% RH (Perkins-Veazie, 2004a). Decay and fruit softening can be reduced with controlled atmosphere storage at 10–20% CO_2 with 5–10% O_2. Fruit are not sensitive to chilling. Ethylene production varies with cultivar and ripeness. Elevated ethylene levels can stimulate the growth of gray mold (*B. cinerea*).

BLUEBERRY Blueberries (*Vaccinium* spp.) are perennials grown for their blue-skinned fruit which encloses a creamy-white to green colored, juicy flesh. A waxy bloom gives the fruit their light blue color. The three major types of blueberries are: (i) lowbush (*Vaccinium angustifolium*); (ii) highbush (*Vaccinium corymbosum*); and (iii) rabbiteye (*Vaccinium ashei*). Lowbush blueberries are small (<1 g per fruit), grow in native stands, and are highly prized for processed products. Highbush blueberries are grown in the mid-latitudes while rabbiteyes are grown in lower latitudes due to their lack of winter hardiness. Rabbiteye fruit often have a gritty mouth feel from seeds and stone cells, but have more anthocyanins than highbush or lowbush and a much longer shelf life than either of them (Perkins-Veazie, 2004b). High quality fruit are harvested when the entire fruit is a uniform blue color. It should be firm with little or no red coloration at the stem end and free of defects. Fruit should be rapidly cooled to <5°C and stored at –0.5–0°C with >90% RH, where they will last for 2 weeks (lowbush and highbush) or 4 weeks (rabbiteye). Fruit deterioration can be delayed and storage life increased to up to 6 weeks with controlled atmospheres of 10–15% CO_2 plus 1–10% O_2 at <5°C. Blueberries are not chilling sensitive. Ethylene production varies with cultivar and gray mold (*B. cinerea*) growth can be accelerated with high levels of ethylene. The main problems encountered postharvest include water loss with fruit shriveling and rot.

CHERRY (SWEET) Sweet cherry (*Prunus avium*) is a rosaceous tree fruit grown for its small, sweet fruit. The fruit is a drupe and the edible portion is the ripened exocarp (skin) and mesocarp (flesh) of the ovary wall which surrounds the inedible endocarp (pit) which contains the seed. Cherries are dark red ('Bing'), light red ('Sweetheart') or yellow with a red blush ('Rainier'). High quality fruit is harvested mature and ripening and harvest index is primarily based on skin color (Mattheis and Fellman, 2004). Fruit must be cooled quickly and stored at –1–0°C at >95% RH where they will last from 2 to 4 weeks. Both controlled atmosphere

and modified atmospheres can prolong storage life when applied to fruit that is not too mature. Fruit is stored at 1–5% O_2 at 5–20% CO_2 in controlled atmospheres and 5–10% O_2 with 5–15% CO_2 with MAP. Maintaining a temperature of 0–5°C is critical for MAP to prevent anaerobiosis. Cherries are not sensitive to chilling and produce little ethylene. However, exposure to exogenous ethylene leads to increased respiration and rapid quality loss. Cherries are prone to a number of postharvest disorders including pitting, bruising, and brown stem. Pitting of fruit is caused by tissue deterioration just below the skin. Bruising results from rough handling and brown stems develop from pedicels scraped during handling. All three maladies may not develop or may not be noticed until fruit reaches the consumer. Gentle handling and proper temperature and humidity control after harvest helps to minimize these problems. Cherries are extremely susceptible to postharvest decay, often by organisms that infect the fruit early in its development. Various pathogens are involved in cherry decay and may include one or more of the following organisms: blue mold (*Penicillium expansum*), gray mold (*B. cinerea*), *Alternaria* sp., brown rot (*Monilinia fructicola*), Rhizopus rot (*Rhizopus stolonifer*), *Cladosporium* sp., and *Aspergillus niger*. Fruit may split as they ripen, making them prone to infection just prior to harvest as well.

CITRUS (GRAPEFRUIT, LEMON, LIME, ORANGE) The fruit of *Citrus* species is a hesperidium, a modified berry that has a leathery, inedible rind that surrounds the edible segments that are filled with juice vesicles (Burns, 2004a, b). All citrus produce little ethylene and are non-climacteric with no postharvest ripening stage.

Grapefruit (*Citrus* × *paradisi*) are available in white and red cultivars. High quality fruit should be turgid with a smooth, blemish-free peel and a pleasant balance of SSC and acidity. When production regions experience warm night temperatures, grapefruit are exposed to 1–5 µl/l ethylene for 12 h to 3 days at 20–29°C depending on production region, to induce the destruction of chlorophyll in the peel. This process is called degreening. Degreening must take place under high RH (90–95%) and air exchange in the degreening room must ensure that CO_2 does not build up. After harvest and any necessary degreening, fruit should be stored at 5–8°C at 95% RH to minimize water loss and peel pitting around the oil glands. Under these conditions fruit will last up to 6 weeks with no decrease in quality. Waxes may be applied to fruit to minimize water loss. Controlled atmosphere storage of grapefruit is not common. Grapefruit exhibit chilling injury symptoms when exposed to temperatures lower than 5°C. Peel pitting not targeted around the oil glands is a classic symptom of chilling injury. Coating grapefruit with high-shine water wax can reduce chilling injury. Intermittent warming or stepwise lowering of the storage temperature can also reduce chilling injury. Granulation, drying of grapefruit segments, is common in late-harvested, larger fruit, especially those stored for long periods. If large fruit are harvested early in the season, granulation is less likely. Oleocellosis is a rind injury caused by rough-handling-induced disruption of oil glands. In particular, excessive force used during harvest or harvesting when fruit is particularly turgid (early in the morning or at high RH), can cause this problem. Symptoms often appear during storage as greenish or brown, firm irregular patches on the rind that soon darken and become sunken. Losses in storage caused by decay can be extensive. Decay may come from infection of fruits by organisms prior to harvest, for example anthracnose (*Colletotrichum gloeosporioides*) or brown rot (*Phytopthora citrophthora*), or by infection of wounds incurred during harvest and handling, such as green and blue mold (*Penicillium digitatum* and *Penicillium italicum*, respectively) and sour rot (*G. candidum*).

Lemons (*Citrus limon*) are grown for their tart, refreshing fruit that are used to enhance the flavor of many foods and to make refreshing beverages. High quality lemons should be uniformly well-colored, smooth, firm, with no defects and have a pleasant citrus aroma. Lemons are harvested when they have a juice content of 28–30% by volume (Gross and Smilanick, 2004). Lemons harvested green have a much longer storage life than those harvested yellow. Lemons should be stored at 7–12°C at between 85 and 95% RH with ventilation and away from products with strong odors, as lemons easily absorb them. Storage life varies with maturity at harvest, season, production area and specific storage conditions but can be up to 6 months. Controlled atmosphere storage is rarely used for lemons. Lemons are sensitive to chilling and should not be held at temperatures less than 10°C, although 3–4 weeks at 3–5°C during marketing is not harmful. Symptoms of chilling injury include skin pitting, interior discoloration, red blotch (a superficial

browning of the rind), and loss of juice. Lemons produce little ethylene and exposure to exogenous ethylene can lead to accelerated quality deterioration during storage. Lemons harvested green are degreened by exposing fruit to 1–10 µl/l ethylene for 1–3 days at 20–25°C. Exposure of lemons to ethylene for degreening also accelerates quality loss.

Some postharvest physiological problems observed in lemons include oleocellosis (see grapefruit discussion), peteca, and membrane stain. Peteca is a pitting of the rind which begins in the white portion and develops into sunken, brown pits on the surface of the rind. Peteca appears to be caused by an imbalance of calcium and potassium in the rind. Membrane stain is a brown discoloration of the membranes between fruit segments and can be avoided by never storing fruit of susceptible cultivars below 13°C. Lemons are susceptible to the same decay-causing organisms as grapefruit.

Limes are divided into Persian limes (*Citrus latifolia*) and key limes (*Citrus aurantifolia*), with Persion limes being nearly seedless and key limes containing numerous seeds (Burns, 2004b). High quality limes should be turgid, appropriately sized, and colored a deep green (Persian limes) or greenish yellow (key limes) with at least 42% juice by volume. Limes are stored at 10°C and 95% RH for up to 8 weeks. Controlled atmospheres are not used for lime storage. Fruit produce little ethylene but are sensitive to exogenous ethylene showing loss of green color and accelerated quality decline. Fruit are susceptible to chilling injury exhibiting peel pitting. Oleocellosis can occur if fruit are harvested when fruit are very turgid. A major postharvest problem with limes is stylar-end breakdown, a general deterioration of fruit tissue integrity at the stylar end, often aggravated by high field heat or rough handling and often observed in larger fruit. Fruit are also susceptible to postharvest decay as described for grapefruit. Stem end rots may occur in both key limes (caused by *Diplodia natalensis*) and Persian limes (caused by *D. natalensis*, *Phomopsis citri*, and *Alternaria citri*).

Sweet oranges (*Citrus sinensis*) come in a wide variety of shape, sizes, and colors (Ritenour, 2004). Fruit may be spherical or oblong, seedless or seeded, green or light to dark orange in color. There are four generally recognized groups of sweet orange cultivars: (i) round; (ii) navel; (iii) blood; and (iv) acidless. High quality fruit should be mature, with a good, uniform color, smooth, and free of defects and decay. Maturity indices vary with region of production but generally include percentage color, SSC, TA, and sugar:acid ratio. Some indices also include a percentage juice by volume. While most commercial operations do not rapidly cool harvested oranges, doing so would improve fruit quality at the market. Most oranges are stored 'on the tree' and harvested as needed for the market. Fruit can be stored at 0–8°C at 85–95% RH for up to 12 weeks after harvest. Specific storage conditions and length of storage vary, depending on maturity at harvest, growing region, and cultivar.

Controlled atmosphere storage can prolong the maintenance of quality in oranges but is not often used because it does not reduce losses due to decay, the number one postharvest problem in oranges. Chilling injury may or may not be a problem depending on production region. For example, oranges produced in Florida or Texas rarely show chilling injury symptoms, while those grown in California or Arizona develop symptoms below 0–5°C. Symptoms include peel pitting, brown staining (external brown patches on the rind), and watery breakdown. Watery breakdown causes the fruit to look like it has been frozen then thawed and may develop several days after removal from storage. Oranges may be degreened by exposure to 5 µl/l ethylene gas for 1–3 days at 20–28°C.

Creasing of oranges is a postharvest deterioration of the white part of the rind (albedo) with the collapse of the overlying colored portion of the rind (flavedo) which leads to the appearance of creases on the skin of the fruit. Creases may split allowing decay organisms to enter the fruit. Thinskinned, mature fruit are susceptible to this condition. Granulation, pitting, and oleocellosis (see grapefruit) are also major problems in orange. Physiologically over-mature fruit are subject to rind staining, the development of brown blemishes from abrasions on the skin. Stem-end rind breakdown appears as the breakdown of tissues at the stem end and is characterized by a small ring of unaffected tissue immediately surrounding the stem scar. Water loss from the fruit between harvest and storage or processing appears to cause this problem, thus rapid movement through packing or processing helps to alleviate this condition. Oranges are susceptible to postharvest decay by a number of organisms, making it the number one postharvest problem. Problems include green mold (*Penicillium digitatum*), blue mold (*Penicillium italicum*), Diplodia stem-end rot (*Diplodia natalensis*),

Phomopsis stem-end rot (*Phomopsis citri*), brown rot (*Phytophthora citrophthora*), sour rot (*G. candidum*), and anthracnose (*C. gloeosporioides*).

CRANBERRY The cranberry (*Vaccinium macrocarpon*) is a perennial, woody, creeping evergreen related to the blueberry. Native to eastern North America, it produces a tart-flavored red fruit often processed into a beverage, dried to mimic raisins (craisins), or canned as a jellied condiment. Ninety-eight percent of the world's cranberries are produced in North America. High quality fruit are harvested as red as possible without over-maturity since only fruit with 75% red color are considered acceptable (Prange, 2004b). Cranberries are stored at 2–5°C at 90–95% RH for up to 4 months. Red color can be enhanced by holding fruit at 7–10°C for a few weeks before cooling. Controlled atmospheres are not normally used for cranberry storage. Cranberries are sensitive to chilling as storage at temperatures near 0°C leads to a rubbery texture and a dull appearance with increased decay. If fruit must be held at lower temperatures, intermittent warming to 21°C for a day every 30 days helps to alleviate some of the chilling injury. Cranberries produce little ethylene. Exposure to exogenous ethylene increases anthocyanin content, especially if fruit are exposed to light during exposure. Physiological breakdown of cranberries can occur in over-mature or senescing berries and is observed as a loss of sheen on the fruit, development of a rubbery texture, and diffusion of anthocyanin throughout the berry tissue.

CURRANT, GOOSEBERRY, AND ELDERBERRY Currants and gooseberries (*Ribes* spp.; gooseberries are sometimes identified as a separate genus, *Grossularia*) are closely related deciduous bushes bearing small berries that are mostly used for processing (Prange, 2004c). Currants may be red, white or pink (*Ribes sativum*) or black (*Ribes nigrum*). Gooseberries are greenish yellow to pink to very dark, nearly black, depending on cultivar and ripeness at harvest. Elderberries (*Sambucus canadensis* L.) are mostly harvested from wild stands with only limited commercial production. The small, bluish-black berries are processed since uncooked fruit is quite astringent and inedible. Currants and elderberries are produced in clusters and high quality fruit should be harvested as ripe (determined by color), large, uniform clusters, free of decay or injury. Black currants may be harvested as individual berries since the cluster does not ripen uniformly. Gooseberries are harvested as single berries either immature (very firm and tart) or ripe (soft and often very sweet), depending on final use. Elderberries ripen over time and must be harvested over a 2 week period in late summer. Fruit should be quickly cooled after harvest and stored at –0.5–0°C at 95% RH for 1.5, 2.5, and 3 weeks for black currant, red currant, and gooseberry, respectively. All three berries are not chilling sensitive. Red currants and gooseberries can be stored under controlled atmospheres of 10–20% CO_2 and 1.5–2% O_2 (depending on species and cultivar) at 1°C to prolong shelf life up to 8–14 weeks. Black currant does not respond to controlled atmosphere storage and no information is available for elderberry.

GRAPES (TABLE AND AMERICAN) The table grape (*Vitus vinifera*) is grown for berries that are harvested in clusters when a minimum SSC is reached and acceptable cultivar-dependent color has developed (Crisosto and Smilanick, 2004). 'Thompson Seedless' is probably the most well-known table grape cultivar. The American grape (*Vitis labrusca*) is grown in regions where *V. vinifera* grapes will not survive low winter temperatures (Perkins-Veazie, 2004d). 'Concord' is a well-known cultivar, along with 'Catawba', 'Delaware', 'Niagara', 'Venus', 'Himrod', and 'Reliance'.

Table grape berries should be firm and clusters well filled with a minimal, cultivar- and market-dependent SSC. Fruit should be cooled as quickly as possible to –1–0°C and SO_2 (100 µl/l for 1 h to control gray mold during storage) applied. Pads soaked in sodium metabisulfite are often used in packing flats to generate additional SO_2 during storage and shipping. Fruit should be stored with moderate airflow (20–40 ft^3/min/t of grapes) at 90–95% RH. Well-handled fruit can be stored for 1 to several months. Controlled atmospheres are not used for storing table grapes, they are not chilling sensitive, produce little ethylene, and are not sensitive to exogenous ethylene.

A major postharvest problem of table grapes is berry shattering, or abscission of individual berries from the cluster. Many table grapes are treated with gibberellic acid at fruit set which weakens the pedicel-berry bond, making the cluster more susceptible to shattering. Rough handling during harvest, packing and shipping increases the incidence of shattering.

Another postharvest disorder of table grapes is called waterberry. It develops soon after verasion (color change of the berries during ripening) as small dark spots on the cluster stem. Berries soon become watery and soft. Waterberry seems to be related to excessive nitrogen fertilization, canopy shading or cool weather during ripening.

GRAPE (MUSCADINE) Muscadine grapes (*Vitis rotundifolia*) are grown in the south-eastern USA and differ from table and American grapes in that they grow singly or in small clusters and are harvested as individual berries, have larger berries, and are uniquely flavored. Berries are harvested when they readily detach from the stem and have an SSC of 14–18% (Perkins-Veazie, 2004e). Fruit are either bronze colored or black. Fruit are cooled as quickly as possible and stored at –0.5–0°C at >90% RH for 1–4 weeks. Muscadines are not chilling sensitive, produce little ethylene, and are not affected by exogenous ethylene. Gray mold (*B. cinerea*) growth may be stimulated by ethylene.

KIWIFRUIT There are a number of edible kiwifruit species worldwide, but by far the most common edible species is *Actinidia deliciosa* (Rushing, 2004c). This is the common egg-sized, fuzzy brown-skinned, green-fleshed kiwifruit. The fruit is a berry with hundreds of dark black seeds embedded in the bright green flesh. A yellow-fleshed fruit of *A. chinensis* is becoming popular as well. High quality kiwifruit at harvest should be firm, free of defects and have a minimum of 6.5% SSC. After harvest, many kiwifruit are cured for up to 48 h at ambient temperatures to allow the stem scar to dry in an attempt to minimize decay in storage. Once cured, fruit are stored at 0°C at 90–95% RH for up to 5 months. Chilling injury has been reported at temperatures near 0°C. Symptoms include a zone of water-soaked tissue in the outer pericarp at the stylar end of the fruit, skin pitting, and scalding. Curing fruit before storage seems to alleviate the occurrence of chilling injury. Kiwifruit are well suited for controlled atmosphere storage at 1–2% O_2 plus 3–5% CO_2 at 0°C which prolongs storage life to 6 months without the fruit softening often experienced with cold storage in air. Mature, non-ripe kiwifruit produce very little ethylene, however, fruit are extremely sensitive to exogenous ethylene at levels as low as 5 parts per billion. This low level of ethylene can induce softening without ripening. Higher levels of ethylene induces ripening, thus storage facilities must be kept free of any ethylene gas. Kiwifruit suffers from a postharvest disorder called hard core, which occurs when the flesh softens rapidly and appears water soaked during storage but the core remains hard and tough. Hard core may be caused by high levels of CO_2 coupled with exposure to ethylene. Ethylene in controlled atmosphere storage can induce distinct white patches in core tissues that are very obvious in ripe fruit. This problem may develop as soon as 3 weeks after harvest. Kiwifruit are susceptible to gray mold (*B. cineria*), blue mold (*Penicillium expansum*), and Phompsis rot (*Phomopsis actinidiae*).

MELONS

WINTER MELONS Winter melons (*Cucumis melo* Inodorus Group) include honey dew, casaba, crenshaw, and canary melons grown for their sweet, melting or crisp, white, light green or pink flesh. All melon types should be firm, well sized and shaped, and free from defects when harvested (Lester and Shellie, 2004). Honey dew melons should be harvested when fruit have a waxy appearance and is whitish to very light green in color. Standard honey dew melons do not slip (abscise with gentle force) when ripe and are cut from the vine when mature, but not necessarily ripe. Hybrid honey dews will slip from the vine and are mostly harvested when ripe. Casaba melons are harvested when fruit are very furrowed and yellow and the blossom end yields to slight pressure. Crenshaw melons are ripe when half of the dark-green skin has turned yellow, the blossom end yields with light pressure and a pleasant aroma is released at room temperature. Fully yellow-skinned crenshaw melons are overripe and not pleasant to eat. Canary melons are ripe when the skin is bright yellow and the blossom end yields to slight pressure. The flesh is crisp and fragrant when ripe.

Honey dew melons cut from the vine and all other mature, but not ripe, melons in this group are not cooled after harvest and are stored at 10°C at 90–95% RH for up to 3 weeks. Honey dews that are harvested full slip, those harvested cut from the vine and induced to ripen with ethylene, and all other ripe melons can be held at 7°C at 95% RH for 7–10 days. All mature, but not ripe, melons produce little ethylene, but benefit from a treatment with ethylene after harvest to induce ripening and promote higher quality. Mature, but not ripe, melons are susceptible to chilling injury at 7°C and sensitivity decreases as fruit ripens. Chilling injury

appears as pitting or elongated lesions on the rind. All melons are susceptible to bruising and compression injury, thus they should not be bulk stored, but rather packed in smaller, suitable containers.

NETTED MELONS Muskmelons (*Cucumis melo* Reticulatus Group) are grown for their orange, melting, fragrant sweet flesh that is surrounded by a rind with a raised netted epidermis. Cantaloupes (*Cucumis melo* Cantaloupensis Group) are non-netted and much less common than muskmelons (Shellie and Lester, 2004). Muskmelons are often categorized as Western shipper melons, grown primarily in the south-western USA, and Eastern Choice melons, grown for local consumption, generally in the eastern USA. Eastern Choice melons are highly sutured while Western shipper melons are not. French Charentais melons are small, round, gray-green skinned, slightly netted melons with prominent dark-green longitudinal stripes. Galia melons have a very fine uniform netting and green flesh. Ananas melons have sparse cracked netting with white, very sweet flesh. Persian melons are very similar to Western shipper melons, but are much larger and have an orange-pink flesh.

All melons in this group must be harvested sufficiently mature to ensure ripening after harvest. Maturity is judged by skin under color and stem slipping. Most of these melons are harvested when half of the stem attaching the melon to the vine has separated from the melon. This is called the half-slip stage. These melons are never cut-harvested as they will not soften or develop sufficient aroma and sweetness if harvested before half slip. Netted melons are cooled after harvest and stored at 2–7°C at 95% RH for 10–14 days. Chilling injury may develop in mature unripe fruit at <2°C. Symptoms include rind pitting and a failure to ripen properly when moved to room temperature. Sensitivity to chilling injury decreases as fruit ripen. Netted melons are climacteric and begin producing elevated levels of ethylene around 4 days prior to stem slip continuing up until about 10 days after harvest. Exposure to exogenous ethylene should be avoided as it reduces storage life. Melons harvested prematurely cannot be induced to ripen with ethylene. Melons are susceptible to sunburn which can cause a bronze coloration of rind under color and net discoloration. Melons are also susceptible to bruising and compression injury.

WATERMELON Watermelon (*Citrullus lanatus*) is a cucurbit producing fruit which come in a wide variety of shapes, sizes, and flesh colors. Fruit may be small and round to large and oblong with light green to almost black skin that may or may not be marbled or striped. Flesh colors may be dark red, pink, yellow and orange and fruit may be seeded or seedless. The fruit consists of a thin rind with a white-fleshed inner rind that is about 2 cm thick, all enclosing the edible flesh which should be crisp, sweet, highly flavored and juicy (Rushing, 2004a). Watermelons should be harvested ripe. Indices for harvest include: (i) a change in the ground color from white to yellow; (ii) drying of the tendril(s) opposite the fruit; and (iii) a change in the rind appearance from glossy to dull. Ripe melons also produce a dull, hollow sound when thumped compared with a brighter, more metallic ring of unripe fruit. In general, watermelons are not pre-cooled and are stored at 10–15°C at 90% RH for 2–3 weeks. Chilling injury of watermelons can occur at temperatures <10°C and appear as rind staining, rind pitting, loss of flesh color and flavor, and increased decay once returned to non-chilling temperatures. Watermelons produce very little ethylene and are extremely sensitive to exogenous ethylene. As little as 5 ppm ethylene can induce rind softening, rind thinning, flesh color fading, and mealiness.

OLIVE Olives (*Olea europa*) are grown for their small, oily drupe, of which the fleshy mesocarp is consumed or pressed to extract the oil (Crisosto and Kader, 2004d). Fruit may be harvested mature green or ripe (purple turning black) for processing as fermented olives or oil extraction. Ripe olives generally have an oil content of 12–25% depending on the cultivar. Green fruit are harvested when they achieve a uniform color with very few white lenticels showing and they extrude a white juice when squeezed. Ripe olives are harvested based on skin color and removal force generally 3–4 months after green olives are harvested. Over-ripe fruit bruise easily and are subject to decay. Fruit sunburn easily and should be protected after harvest. Ripe fruit should be processed immediately. Fruit can be stored for a short time at 5–7.5°C with 90–95% RH. Storage in a controlled atmosphere consisting of 2–3% O_2 with 0–1% CO_2 can prolong storage of fresh green olives up to 12 weeks. Green olives are subject to chilling injury at <5°C. Injury symptoms appear as a brownish discoloration near

the pit which becomes more intense with time and progresses through the rest of the flesh until the fruit eventually looks like it has been cooked. Both green and ripe olives produce little ethylene. Both are susceptible to injury from exogenous ethylene which is manifest as a loss of green color in green olives and a loss of flesh firmness in either type. Nailhead is a postharvest disorder caused by storage of fruit at 10°C for >6 weeks or at 7.5°C for >12 weeks. It appears as surface pitting and spotting.

PEACH AND NECTARINE Peaches (*Prunus persica*), native to China and Persia, are one of the 'summer fruits' prized for their sweet, tender flesh with a bright, fresh flavor. Nectarines (*Prunus persica*) were probably derived from peaches and are grown nearly anywhere peaches are grown (Crisosto and Kader, 2004a, b). The best peaches and nectarines have a high sugar content (at least 11% SSC) with slight acidity (less that 0.7% TA) (Crisosto and Kader, 2004a, b). Nectarines have slightly higher TA than peaches. Since they are rather delicate when ripe, fruit are harvested mature and allowed to fully ripen off the tree. Harvest is based on a change in skin ground color from green to yellowish. Fruit firmness is also a measure used to determine 'firm mature' fruit ready for harvest (27–36 N/cm^2) or 'tree ripe' fruit (9–14 N/cm^2) and varies considerably among cultivars. Fruit should be cooled as quickly after harvest as possible and cooled to –1–0°C with 90–95% RH with good ventilation where they will store for 2–4 weeks with minimal loss in quality. Color retention and fruit firmness can be enhanced with controlled atmosphere storage of 6% O_2 plus 17% CO_2 at 0°C. Peaches are climacteric, thus ethylene evolution depends on ripening stage. Fruit are sensitive to exogenous ethylene, as it will induce ripening in mature fruit.

Peaches are susceptible to chilling injury at all recommended storage temperatures, but especially if stored at 2.2–7.6°C, thus they should be marketed as quickly as possible. Chilling injury appears as internal breakdown of the flesh. Internal breakdown seems to affect nectarines less than peaches, however, it is still a major postharvest problem. Internal breakdown is characterized as dry, mealy or woolly flesh or translucent flesh radiating from the pit. Flesh may alternately be hard textured. Intensely red-colored flesh radiating away from the pit ('bleeding') is another symptom. Flavor is often compromised before any symptoms are visible. Another postharvest problem called 'inking' is a superficial skin discoloration caused by abrasions and heavy metal contamination (iron, copper, aluminum) of cooling or wash water which appears 24–48 h after harvest. Peaches are susceptible to postharvest rots, particularly brown rot, caused by *M. fructicola*. Infection begins during flowering and rot may occur anytime thereafter. Fruit are also susceptible to gray mold (*B. cinerea*) and Rhizopus rot (*R. stolonifer*).

PEAR (EUROPEAN) The European pear (*Pyrus communis*) is a member of the family *Rosaceae*. The edible portion of the fruit is the enlarged, fused bases of the calyx, corolla, and stamens. Pears are harvested mature and must be exposed to 2–8 weeks of cold storage at –1°C to induce ripening upon removal to 20°C. The length of cold storage needed to induce ripening at 20°C varies with cultivar and ripening will occur in 4–7 days at 21°C. Fruit that is harvested before they are mature are susceptible to superficial scald, water loss, and skin discoloration due to rubbing against other fruit or the storage container. Superficial scald does not appear in storage, but rather is a patchy discoloration of the skin which occurs after removal from storage for ripening. Eating quality is not affected, however, the fruit are visually unappealing. Fruit harvested 'over-mature' tend to develop core breakdown and CO_2 injury. Fruit firmness is the best indicator of maturity (Chen, 2004). Heat unit accumulation during 9 weeks following bloom provides a good estimate of harvest date. Fruit are extremely sensitive to storage temperature with fruit stored at –1°C lasting 30–40% longer in storage than those stored at 0°C. Fruit should be stored at >90% RH to prevent dehydration. Controlled atmosphere storage at 2–2.5% O_2 with 0.8–1% CO_2 can prolong storage life. Once appropriately chilled pears are brought to ripening temperatures, autocatalytic ethylene production commences and ripening occurs. If fruit are not chilled correctly, ethylene production will not commence and fruit will fail to ripen. Inadequately chilled fruit may be induced to ripen if exposed to exogenous ethylene.

Pithy brown core is a postharvest malady affecting 'd'Anjou' pears exposed to elevated CO_2 levels. Pithy, brown areas develop near the core of the fruit and may extend into the surrounding flesh. The tissues are dry and pithy, not soft and watery like that which develops from core breakdown. Core breakdown is characterized by softening and

tissue breakdown near the core. Initially the tissue is soft and watery but eventually turns brown. Low O_2 levels in controlled atmosphere storage can lead to brown speck in 'd'Anjou' pears. It appears as brown specks on the skin. Senescent scald may affect any pear cultivar. It develops in cultivars that senesce during storage which leads to an inability to ripen once removed from storage. Additionally, yellow, then brown discoloration of the skin occurs, the fruit are inedible, will not ripen, and the skin easily slips off the fruit. To prevent senescent scald, fruit must be removed from storage before it begins to develop.

PINEAPPLE The pineapple (*Ananas comosus*) is a tropical, multiple, aggregate fruit. High quality fruit should have flat eyes (the individual fruitlets), and fresh, green crown leaves. Fruit is harvested based on eye flatness and SSC which should be at least 12% (Paull and Chen, 2004a). Consumers also look for nicely colored fruit (yellow ground color with slight green over color) and rich, fruity aroma. Fruit do not ripen once harvested. Fruit are susceptible to chilling injury below about 7°C and are generally stored between 7 and 12°C at 85–95% RH for 7–10 days. A major symptom of chilling injury is internal browning. Fruit are susceptible to sunburn in the field and to bruising at any point during handling. Fruit produce little ethylene and are generally insensitive to exogenous ethylene exposure.

PLUM AND FRESH PRUNE Japanese plums (*Prunus salicina*) are usually eaten fresh or as jam or jelly while European plums (*Prunus domestica*) may be eaten fresh but are most often dried whole into prunes (Crisosto and Kader, 2004c). High quality fruit of either type of plum has a high SSC, acceptable TA, and minimal astringency. Plums are often harvested based on skin color changes, increases in SSC and a decrease in flesh firmness. Plums should be cooled to –1–0°C and stored at 90–95% RH under well-ventilated conditions where they will last for 4–6 weeks. Controlled atmosphere storage at 1–2% O_2 and 3–5% CO_2 helps prevent flesh softening and undesirable changes in ground color. Most plums are susceptible to chilling injury when stored at 5°C but not when stored at 0°C (Crisosto and Kader, 2004c). Chilling injury symptoms usually appear when fruit is moved to warmer temperatures for ripening and appears as translucent flesh which soon browns. Lack of juiciness may also develop in late-season cultivars. Flesh translucency is also called gel breakdown. Ethylene gas production depends on the stage of ripeness and exogenous ethylene will induce ripening in all plums. Internal browning not associated with chilling injury is caused by high temperatures during fruit maturation and delayed harvest. Plums are susceptible to postharvest rots such as brown rot (*M. fructicola*), gray mold (*B. cinerea*), and Rhizopus rot (*R. stolonifer*).

POMEGRANATE The pomegranate (*Punica granatum*), also called the Chinese apple, requires a long, hot summer for production. It is very tolerant of cold, drought and salt stress (Pekmezci and Erkan, 2004). The fruit is round with a prominent calyx and a hard, leathery skin. A bright red pulp (aril) surrounding the edible seed is consumed directly or processed into juice. The white, leathery membrane separating layers of seeds is not edible and may be somewhat bitter and astringent. High quality fruit must have a blemish-free, nicely colored skin and juicy arils surrounding small seeds. All pomegranates are harvested fully ripe. The acid:sugar ratio of the juice must meet market requirements and varies with cultivar. Pomegranates are 45–65% juice and the skin contains up to 30% tannins which can be used in medical or dye industries. TA varies with cultivar: (i) <1% in sweet cultivars; (ii) between 1 and 2% in sweet-sour cultivars; and (iii) >2% in sour cultivars. SSC ranges from 8 to 20% depending on cultivar. Pomegranates are susceptible to chilling injury below 5°C and most cultivars are stored at around 6°C at 90–98% RH to prevent dehydration. Chilling injury is observed as pitting and brown discoloration of the rind, brown discoloration of the membrane separating arils, and pale-colored arils. A controlled atmosphere of 3% O_2 with 6% CO_2 reduces loss of TA and vitamin C. Fruit can be stored for up to 6 months. Fruit produce little ethylene and are not sensitive to exogenous ethylene.

PUMPKIN AND WINTER SQUASH There are three species in the family *Cucurbitaceae* that are grown for their fruit which is harvested physiologically mature: (i) *Cucurbita pepo* (pumpkin and acorn squash); (ii) *Cucurbita maxima* (winter squash and giant pumpkin); and (iii) *Cucurbita moschata* (butternut squash, crookneck squash, and calabaza) (Brecht, 2004a). Fruit are harvested physiologically mature in the fall and many can be stored

for many months with no loss in quality. Fruit are harvested when the rind has become hard and has lost is sheen, the fruit has just begun to abscise from the plant, the ground spot of the fruit has become yellow, and tendrils nearest the fruit on the vine have turned brown. Fruit should be harvested when mature, based on these characteristics and not based on vine death. Quality improves more in storage than on the vine once fruit is mature.

Fruit are stored for several months at 10–13°C at 50–70% RH with good ventilation. Chilling injury can occur at temperatures <10°C and appears as skin pitting and a loss of flavor as well as accelerated development of rots when returned to non-chilling temperatures. Fruit produce little ethylene. Exposure to exogenous ethylene may lead to yellowing of green fruit and stem abscission. While curing of pumpkins and winter squash at 24–27°C for 10–20 days before storage to harden the rind is often recommended by some authorities, it is not beneficial and may actually reduce eating quality of some cultivars (Brecht, 2004a).

RASPBERRY Raspberries (*Rubus ideaus*) are soft, sweet, juicy succulent fruits that are grown in many areas of the world. While their root systems are perennial, their fruit-bearing shoots are either annual (primocane fruiting cultivars) or biennial (floricane fruiting cultivars). Raspberries are available in red, yellow, black, and purple cultivars. Red and yellow raspberries are divided into two subspecies, *Rubus ideaus* subsp. *vulgatus*, the European red raspberry, and *Rubus ideaus* subsp. *strigosis*, the American red raspberry. Black raspberry cultivars may be *Rubus occidentalis* (North American black raspberry) or *Rubus glaucus* (a South American tetraploid black raspberry). Purple raspberries (*Rubus neglectus*) are hybrids of red and black raspberries (Perkins-Veazie, 2004c). Fruits are composed of numerous drupelets attached to a common receptacle which remains on the plant when fruit is harvested. Harvested fruit is thimble-shaped with a hollow center, making it extremely delicate.

High quality fruit are fully colored, regularly shaped, firm and free of defects and decay. Sunscald (individual whitish drupelets) caused by excessive UV radiation can be a problem in raspberries. Fruit are harvested ripe and are easily removed from the plant with little force when ready to pick. Fruit should be cooled rapidly and are stored for no more than 2–5 days at –0.5–0°C at >90% RH. Raspberry deterioration is reduced under controlled atmosphere storage in 10–20% CO_2 with 5–10% O_2. Levels of CO_2 greater than 20% can induce softening, discoloration, and development of off-flavors. Raspberries are not chilling sensitive. Ethylene production varies with cultivar and exposure to exogenous ethylene can stimulate gray mold (*B. cinerea*) growth and cause darkening of red raspberries.

STRAWBERRY The strawberry (*Fragaria* × *ananassa*) is a perennial that is often grown as an annual for its fruit which consists of many achenes (the botanical fruit, often called seeds) on a common swollen receptacle that becomes sweet and turns red when ripe (Fig. 16.7). Fruit should be harvested as ripe as possible since fruit does not ripen after harvest (Mitcham, 2004). Harvest of strawberry is solely based on color. Fruit should be firm, well colored, sized and shaped for the cultivar, with the calyx attached at harvest. Fruit must be cooled immediately upon harvest to 0°C and stored at 90–95% RH for up to 7 days. Modified atmospheres of 10–15% CO_2 are often used during shipping to reduce decay and fruit respiration, with whole pallets of fruit covered and treated. Strawberries are not chilling sensitive, produce very little ethylene, and are not sensitive to exogenous ethylene. Reduction in storage-room ethylene may reduce decay by reducing *Botrytis* growth. If >15% CO_2 is used during MAP, skin may take on a bluish caste, fruit flesh may turn white, and off-flavors may develop.

TOMATO The tomato (*Solanum lycopersicum*) is grown worldwide for its fruit (a berry) which is eaten fresh or cooked in various ways. Tomatoes come in many colors (red, yellow, ivory, purple) and shapes (cherry, plum, grape, mini-pear, slicing, and beefsteak). Tomatoes are harvested at any stage from physiologically mature to fully ripe, and should be firm, turgid, uniform in color, and shiny with no defects. Mature fruit can be ripened off the vine. Most tomatoes are field grown, however, significant greenhouse production occurs in Canada, Holland, Spain, and Israel.

It is often difficult to determine the physiological maturity of tomato (Sargent and Moretti, 2004), but it is often based on the color of seeds, amount of gel in the seed locule, and the color of any gel in the seed locule. Only tomatoes that are harvested when all locules contain gel and the seeds are pushed aside when sliced through the equatorial

Fig. 16.7. Strawberries (*Fragaria* × *ananassa*).

plane will ripen to the highest quality. Fruit that are less mature than this stage will not ripen to very good quality. Ripeness stages are often defined by skin-color transformation from totally green to fully red.

Tomatoes should be cooled to 12°C for storage or 20°C for ripening, both at 90–95% RH. Controlled atmosphere storage is feasible with 3% O_2 plus 2% CO_2. Exact storage conditions often depend on cultivar and stage of maturity and ripeness at harvest. Prolonged storage may lead to the production of off-flavors. Fruit are sensitive to chilling at temperatures less than 10°C, depending on the cultivar and the stage of ripeness. Symptoms of chilling injury include surface pitting, non-uniform ripening, and increased storage rots. Tomatoes produce significant amounts of ethylene and are also sensitive to exogenous ethylene, which will induce ripening of mature fruit. Fruit are often ripened with ethylene gas at the marketing stage.

Tomato fruit are susceptible to a number of physiological problems. Blotchy ripening is the development of green and yellow patches on the surface of red tomatoes. Sunburn results from elevated fruit temperatures during development caused by excessive fruit exposure to light during fruit development. This disrupts lycopene synthesis and leads to the development of yellow areas in affected skin and fleshy tissues which remain even after ripening. Blossom end rot results from poor calcium uptake of insufficient translocation into developing fruit. This leads to the development of dry, dark-brown discolored patch at the stylar end of green fruit which eventually is colonized by decay-causing organisms. Graywall is observed as necrotic vascular tissue in the pericarp wall. The problem starts in green fruit and seems to result from trying to grow tomatoes under marginal conditions or to be a response to tobacco mosaic virus or a bacterial infection. Internal bruising caused by rough handling of green fruit is observed as impaired ripening in locular gel and leads to a reduction in vitamin C content, a reduction in acidity, and a reduction in total carotenoids, and an unacceptable consistency with the development of off-flavors. Tomatoes are susceptible to many bacterial and fungal rots that may begin in the field or greenhouse and carry through into storage, thus good sanitation at every stage of processing must be practiced.

Mature or immature fruits

COCONUT The coconut (*Cocos nucifera*) is consumed at two different stages of development. Immature coconuts (6–9 months after flowering) are harvested for their liquid endosperm (coconut water) and jelly-like meat while mature coconuts (12 months after flowering) are harvested for their water as well as their hardened, white endosperm (Paull and Ketsa, 2004). Well-developed nuts are harvested at the appropriate stage for their use. Immature fruit are harvested when the short stem that held the male flower on top of the coconut (called the rachillae or rat's tail) is half browned and the coconut skin around the calyx is creamy white or yellowish. Mature fruit are characterized by a totally brown rat's tail and skin that is turned brown. Both immature and mature coconuts are husked to varying degrees after harvest. The immature nut is partially husked while the entire husk is removed from mature nuts. Mature coconuts with husks intact can be stored at ambient temperatures for 3–5 months before the liquid endosperm has evaporated, the shell has cracked, or the nut has sprouted. Young, husked and wrapped coconuts are held at 3–6°C at 90–95% RH for 3–4 weeks. Unwrapped nuts displayed at ambient temperatures will last 1–2 days. Treatment with sodium metabisulfite reduces browning of young, husked coconuts and may prolong shelf life by several days. Immature coconuts are chilling sensitive, with their green skins turning brown after 7 days at 0°C. Other quality attributes are unaffected by chilling. Coconuts produce very little ethylene and are not sensitive to exposure to exogenous ethylene. Mechanical damage, water loss, and mold growth

are the most common postharvest problems associated with both immature and mature coconuts.

PAPAYA Papaya (*Carica papaya*) is a tropical fruit that can be eaten either green or ripe. High quality fruit should be appropriately sized for a given cultivar, with a smooth skin free from blemishes (Zhou *et al*., 2004). Fruit destined for Western countries should not have the heavy, musky aroma associated with some South-east Asian cultivars. For fresh consumption as a sweet fruit, papayas should be harvested after ripening has commenced and the fruit has at least 11.5% SSC and some skin has yellowed. After harvest, fruit should be cooled and stored at 7–13°C at 90–95% RH. Chilling injury may occur at 7–10°C while fruit continue slow ripening at 10–13°C. Fruit that have just begun to ripen can be stored at 7°C for up to 14 days without chilling injury. Ripe fruit can be held at 1–3°C for a week or so. The more immature the fruit, the greater is its susceptibility to chilling injury, which is manifest as skin scald, water soaking of flesh, and the development of lumps around the vascular tissue in the flesh. Controlled atmosphere storage is not utilized in papaya production. Papayas produce some ethylene as they ripen from the inside out. Exogenous ethylene is not recommended to induce ripening as it causes ripening from the outside inwards, which leads to undesirable excessive fruit softening. Fruit may develop green, slightly sunken areas when ripe that result from abrasion injury to the fruit when green. Small brown, raised skin freckles may develop on sun-exposed fruit after a period of cool, rainy weather. The fruit skin may also show scald if exposed to sun either before or after harvest. Premature ripening may occur in fruit with low calcium levels.

PEPPER Peppers (*Capsicum annuum*) are generally known as bell peppers and chili peppers (González-Aguilar, 2004). Bell peppers are harvested either immature or allowed to mature and ripen turning red, yellow, orange, gold, brown, or purple. Ripe fruit are generally sweeter than immature fruit and often have a less intense characteristic pepper flavor of immature fruit. Chili peppers are varied in shape, size, and color and may be mild to very hot. They are harvested ripe and may be consumed fresh or dried. After harvest, peppers can be stored at 7–13°C at 90–95% RH for 2–3 weeks. Chilling injury occurs at <7°C and appears as surface pitting, seed-cavity discoloration and water soaking of flesh. Ripe peppers are less sensitive to chilling than green peppers. Peppers are non-climacteric and produce little ethylene. Exposure to exogenous ethylene causes flesh softening and increased respiration but not color change. Peppers are susceptible to blossom end rot, similar to that observed in tomatoes, which is caused by a calcium deficiency in the developing fruit.

Floricultural products

Fresh-cut flowers and greens

Many different species are harvest for cut flowers and the greens that often accompany them (Fig. 16.8). Species include ferns and lycopods, angiosperms and gymnosperms (Reid, 2004). Postharvest quality of these products is 100% visual and the harvested commodity often consists of more than one plant organ. For example, a long-stem rose consists of a stem, leaves, and a flower bud, each contributing to the quality of the commodity. Imperfections in one or more of these organs greatly detract from its value. The main characteristics that result in reduced quality of cut flowers and greens include: (i) senescence of flowers and leaves; (ii) wilting; (iii) tropic responses; and (iv) shattering or loss of petals and leaves.

Wilting is a major cause of reduced quality. Wilting usually is caused by the obstruction of the vascular tissue after harvest by bacteria, dirt, or an air embolism. Most cut commodities should be stored at >95% RH at low temperatures to minimize water loss during storage. Sugars released into the vase water from cut stems provide an ideal growing environment for bacteria, yeast, and other fungi. Metabolic products from these organisms or the organisms themselves can plug the xylem of the commodity, effectively preventing uptake of the vase solution, causing wilting. In order to prevent growth of such organisms, care must be taken along the supply chain to prevent contamination of the commodity with these organisms and to prevent the establishment of a favorable environment for their growth and development. Clean water should always be used in making storage solutions and a biocide should be part of the solution recipe. All containers and utensils should be routinely disinfected.

Dissolved minerals in water often make the storage solution alkaline which greatly reduces water uptake by most commodities. Either water free

Fig. 16.8. Sunflowers (*Helianthus annuus*) ready for harvest.

from dissolved minerals or acidified water should be used to ensure that the storage solution is acidic. Citric acid is often used to acidify cut flower solutions. Certain chemicals often found in tap water are often toxic to many cut ornamentals. Sodium, which is often found at high levels in 'softened' water, is toxic to carnations and roses while fluoride, often found in drinking water, is toxic to gaillardia, gerbera, gladiolus, roses, and freesia.

An air embolism is a small bubble of air that becomes trapped in a xylem vessel, preventing water movement. They often occur at the time of cutting. These obstructions can be removed by: (i) cutting stems under water; (ii) ensuring that the storage or vase solution is acidic (pH 3–4); (iii) heating the solution to 40°C or cooling it to 0°C; (iv) placing stems in >20cm of water; or (v) briefly immersing stems (10 min) in a 0.02% (v/v) detergent solution.

Flower and leaf senescence is another major cause of reduced vase life. Leaf yellowing and senescence is also detrimental to quality, especially in crops such as *Alstroemeria, Chrysanthemum*, and marguerite daisy (*Argyranthemum frutescens*) where leaves are an important part of the floral display (Reid, 2004). Shattering of flower petals, leaves, and even branchlets leads to reduced product quality. It is often caused by exposure to ethylene.

One of the main physiological considerations in determining the vase life of cut flowers and greens is their food supply after harvest. Sugars and starch stored in the various commodity parts supply the food needed for continued development (usually flower opening) and metabolic maintenance. Many floricultural crops are harvested early in the day to allow for postharvest processing. This is unfortunate since food reserves are often at their lowest early in the day. To compensate for this, many cut flowers are fed a sugar solution at low temperatures for as long as 24 h immediately after harvest. Leaf yellowing is probably related to a reduced carbohydrate status in the cut commodity.

Spike-type flowers such as gladioli and snapdragon are particularly susceptible to reduced quality from geotropism. To prevent unwanted geotropic bending of flower spikes, always store spikes upright whenever possible. Spikes will bend upwards if stored horizontally.

As with all other horticultural commodities, cut flowers and greens have harvest indices for maximizing storage life. Many cut flowers are harvested in the bud stage, but at a point where the buds are developed enough so that they will fully open upon display. Some are harvested when buds are just starting to open (*Rosa, Gladiolus*) while others are harvested when flowers are nearly fully open

(*Chrysanthemum, Dianthus*). Cut foliage is harvested when the youngest leaf is fully expanded.

Flowers are often graded based on the weight of a given stem length or simply stem length of the cutting. Weight of a given stem length is often positively related to the quality of flower(s) the stem holds. Stem straightness and freedom of the cutting from any defects are also used in grading schemes. Most flowers are sold in bunches of five, ten, 12 or 25 stems. Specialty flowers such as members of the family *Orchidaceae*, and *Zingiber, Strelitzia, Anthurium*, and *Helianthus* are often sold singularly or in smaller bunches.

All cut flowers except those that are chilling sensitive (*Anthurium, Zingiber*, tropical orchids, *Strelitzia*) should be cooled as quickly as possible after harvest and stored at 0–1°C. Chilling-sensitive species should be cooled and held at 10°C. Chilling injury may be observed as darkened leaves and petals or water-soaked petals. Most flowers should be stored at 95–99% RH.

Most cut flowers are sensitive to ethylene gas. Exposure to even small amounts of ethylene often hastens senescence. Some flowers produce ethylene as they age and senesce (*Dianthus*) while others produce very little ethylene themselves (*Antirrhinum*) yet are very sensitive to exogenous ethylene and shatter quickly upon exposure to even very low levels of ethylene. Many cut flowers are treated with anionic silver thiosulfate or 1-MCP to inhibit ethylene's effects. Storage at the proper temperature minimizes ethylene production and sensitivity to ethylene.

Controlled atmosphere storage is not normally employed in the cut flower industry (Reid, 2004).

Potted flowering, foliage, and bedding plants

POTTED FLOWERING PLANTS Flowering potted plants are an important commodity in horticulture. Specimens are often greenhouse-grown then shipped to their point of sale, often hundreds, even thousands of miles away from the site of production. Only the highest quality plants should be shipped as they will often have to endure low light, poor ventilation, harmful gases, and excessive vibrations during transit.

Quality of flowering potted plants is based on flower longevity and leaf quality. Maintaining vibrant flowers and green foliage at the same time for an extended period is not an easy task when plants are subject to the stresses of shipping mentioned above. Additionally some species are cold tolerant while others are chilling sensitive. Sensitivity to ethylene also varies considerably among species.

Production practices may greatly influence flower longevity and leaf color. Excessive fertility leads to poor flower longevity. For example, *Chrysanthemum* fertilization is halted 3 weeks prior to marketing with a concomitant increase in flower longevity of 7–11 days (Nell, 2004a). Excessive watering during the final 2 weeks of potted rose (*Rosa* spp.) production leads to significant losses during shipment due to damage to the root system.

During shipping losses can occur due to: (i) disease; (ii) incorrect temperature; (iii) exposure to ethylene; and (iv) extended shipping period. Appropriate temperature management during shipping is the single most important factor during shipping determining quality retention. All potted flowering plants should be shipped at the lowest species-appropriate temperature possible. This leads to decreased respiration, ethylene production and consumption of food storage reserves by the plants. Chilling-sensitive plants are shipped at 10–12°C while chilling-insensitive plants are shipped at 2°C.

Exposure to ethylene is also a potential problem. Open flowers are usually more sensitive to ethylene than buds and ethylene sensitivity of the whole plant increases with temperature. Injury symptoms include premature flower senescence, bud drop, and leaf yellowing. Several anti-ethylene chemicals such as 1-MCP and silver thiosulfate can be used to minimize damage, especially in chilling-sensitive species that must be shipped at higher temperatures.

ORCHID *Phalaenopsis* and *Dendrobium* orchids have become enormously popular as flowering potted plants. The major problem associated with the supply chain and handling of orchids is their extreme sensitivity to ethylene (Wang, Y.T., 2004). Pollination and emasculation trigger ethylene evolution which causes rapid wilting and water soaking of flower petals within 3 days, greatly reducing flower longevity. Some success has been achieved in greatly reducing ethylene sensitivity of orchid flowers using treatments with silver thiosulfate and 1-MCP. Protection from ethylene injury can last up to 7 days.

Tropical orchids are also extremely susceptible to chilling injury at temperatures as high as 15°C. Preconditioning plants for 10 days at 25°C followed by 10 days at 20°C may reduce the amount

of injury. Light is important during storage and retailing for maintaining flowering and flower quality in orchids.

FOLIAGE PLANTS A number of different species are grown as potted plants specifically for their foliage (Nell, 2004b). Many of the same problems encountered with potted flowering plants are encountered when shipping potted foliage plants. Plants are shipped in darkness which is not conducive to maintaining healthy foliage. Temperature control during shipping is critical for maintaining healthy, high quality plants. Most foliage plants are shipped at 15–18°C at 85–90% RH. Lower shipping temperatures may lead to chilling injury, especially if transit time is long. Foliage plants should not be shipped with products that emit ethylene (flowers, fruits, and vegetables). Foliage plants are particularly susceptible to ethylene injury during shipping since they are shipped at relatively high temperatures where even extremely low levels of ethylene can induce damage.

Many growers acclimate their plants for shipping by reducing fertilizer and water application, decreasing light levels and temperature for 2–4 weeks before shipping. Acclimatized plants suffer fewer problems during shipping and marketing and have a longer shelf life than non-acclimatized plants.

BEDDING PLANTS Many vegetable transplants and flowering bedding plant transplants must be transported or stored for brief periods. Proper transport and storage are required to prevent problems both to the plant at the transplant stage and to the commodity once it is planted (Kim *et al*., 2004). Transplant death is an obvious problem. Premature or delayed flowering is a major problem observed following improper handling during transport and storage. Some vegetable transplants such as onions (*A. cepa*) may bulb prematurely if handled improperly. Plugs are smaller than transplants and are usually more sensitive to environmental conditions during shipping and storage. However, their small size often makes them easier to handle during shipping and storage.

Storage temperature and irradiance are the two factors that determine the length plug plants can be stored without damage. Light during storage or transport reduces damage especially when storage temperatures are higher and length of storage is longer. Shipping and storage temperature are species dependent. Many plugs benefit from cooling prior to shipping if shipped over long distances. Plug water status should be closely monitored during storage and care must be taken not to overwater plugs as this is likely to cause problems with *Botrytis*, a major problem of plug plants during storage. Lower cooler RH reduces the incidence of *Botrytis* but may increase the frequency of watering needed.

Flower bulbs

Many species that are geophytes (plants with underground storage organs) are important horticultural crops. The storage of their bulbs (a term often used to include bulbs, corms, tubers, rhizomes, tuberous roots, and enlarged hypocotyls; De Hertogh and Le Nard, 2004) is an important part of the production cycle. While there are over 60 taxa grown commercially, six of them account for 90% of the world production of flower bulbs: *Tulipa* (39%), *Narcissus* (20%), *Lilium* (19%), *Gladiolus* (8.5%), *Hyacinthus* (4%), and *Iris* (3%) (De Hertogh and Le Nard, 2004).

Pre-production storage of these propagules is species, sometimes cultivar, specific and local authorities should be consulted for recommendations. This section will consider general storage and pre-production (postharvest) practices and problems.

Flower bulbs are divided into two categories: (i) those that will be used for propagation (planting stock); and (ii) those that will be used for commercial production of cut flowers, flowering potted plants, or planted directly into the landscape (commercial bulbs) (De Hertogh and Le Nard, 2004). For both categories, optimizing pre-production storage and growing conditions combine to achieve a number of objectives:

- to control flowering from floral initiation to anthesis;
- to control disease and insects; and
- to prevent physiological disorders.

An important physiological characteristic to know for each bulb species in question is when does floral initiation occur and what factor(s) influence it. Some bulbs initiate flowers before harvest and placement in storage (hardy *Narcissus*), while others initiate flowers during postharvest storage (*Tulipa* and *Hyacinthus*) or in the production field or greenhouse (Easter lily, *Lilium*). In addition, it is

important to know how long floral development from initiation to anthesis takes. This process may be as short as a few weeks or it may take months. The species temperature sensitivity with respect to flowering is also important information for success. As with other horticultural commodities, it is also important to know the species sensitivity to ethylene, RH requirements during storage, and any other particular storage requirements for a species.

Temperature is undoubtedly the most important factor that must be regulated during the pre-planting phase of production as floral production is precisely controlled by a specific temperature and duration and these values vary considerably among species, from –2 to 34°C for days to weeks (Hartsema, 1961).

Ventilation is important for regulating O_2, CO_2, ethylene, and RH and varies with species and storage temperature. Moisture, and in particular RH, must be monitored and regulated to prevent desiccation but at the same time prevent the growth of disease-causing organisms. Some bulbs are stored in moist peat moss or other material to prevent desiccation. All bulbs except Dutch iris are sensitive to ethylene of 0.1 ppm and higher. Exposure to even extremely low levels of ethylene can cause floral abortion rendering the bulb useless. It is often wise to monitor storage rooms for ethylene, especially ultra-sensitive species such as tulips. Modified atmospheres have been used to a limited extent for bulb storage.

Since storage requirements and subsequent problems associated with improper handling of bulbs during the postharvest/pre-production period are very particular for each species, a general discussion for only the six major genera will follow.

***GLADIOLUS* (GLADIOLI)** Gladioli plants are propagated via corms, short, swollen, vertical underground stems. Flower formation occurs after planting and is dependent on soil temperature (13°C optimal). Corms used for either planting stock or commercial production are treated similarly. After harvest, corms are cleaned and graded then stored for 2–3 weeks at 15–23°C. Corms are then stored at 2°C under highly ventilated conditions for 8–10 weeks to break dormancy. Corms held for long-term storage should be held at 2°C. Prior to planting, corms are stored at 20–30°C for 4–8 weeks to promote sprouting.

***HYACINTHUS* (HYACINTHS)** Hyacinths are propagated via bulbs, short stems with swollen fleshy leaves or leaf bases. Flower development requires at least 10–14 weeks of temperatures between 5 and 9°C after planting. Depending on intended use, hyacinth bulbs are treated differently after harvest.

For production bulbs, bulbs are harvested in June (The Netherlands), cleaned and graded then stored at 30°C under highly ventilated conditions until 1 September. Bulbs are then stored for at 38°C for 2 weeks followed by 44°C for 3 days, then at 25.5°C until planting. Large bulbs used for scooping and scoring are stored at 25.5°C from harvest through to planting. Scooping and scoring are techniques that can be used when propagating bulbs. Scoring consists of making two shallow perpendicular cuts into the basal plate of a bulb to induce callus formation, from which bulblets (baby bulbs) will form. Scooping involves removing most of the basal plate with a spoon or similar instrument, leaving the rim of the basal plate intact. Callus tissue soon forms, followed by bulblets.

Bulbs used for flowering are available in two forms: (i) prepared bulbs; and (ii) regular bulbs. Prepared bulbs are used for very early forcing. After harvest in June, bulbs are stored under dry, highly ventilated conditions at 30°C for 2 weeks, followed by 25.5°C for 3 weeks, then 23°C until the uppermost floret on the inflorescence reaches a particular floral stage called stage A_2. Stage A_2 is when the second whorl of stamens is visible in the developing floret. Bulbs are then stored at 17°C until planting. (Note that floral *initiation* occurs during this warm storage, each temperature promoting a particular aspect of floral initiation and differentiation, but complete floral *development* requires exposure to much lower temperatures after planting.) After planting prepared bulbs require a minimum of 10 weeks at 5–9°C for floral development. Regular bulbs which are used for later forcing are stored at 25.5°C. Four weeks before forcing, bulbs are stored at 17°C for 4 weeks. After planting, regular bulbs require a minimum of 13 weeks at 5–9°C for floral development. Bulbs used in the landscape are harvested then stored at 25.5°C. They are shipped and then subsequently stored at 17°C. The extra care given to prepared bulbs in moving them from 30°C to 25.5°C to 23°C then to 17°C allows them to flower up to 1 month earlier than bulbs that are not prepared.

***IRIS* × *HOLLANDICA* (DUTCH IRIS)** There are many species of iris, however, the Dutch iris (*Iris* × *hollandica*) is the one used extensively for forcing. These iris are propagated via bulbs and should be handled very carefully to prevent mechanical injury which often leads to infection by *Fusarium* and *Penicillium*.

Production bulbs (bulbs for propagation) for planting are handled very carefully to minimize flower formation and produce round commercial-sized bulbs. Bulbs that are in the 7/8 cm size category are extremely sensitive to temperature with respect to flower formation. Larger bulbs readily flower and smaller ones do not and neither are affected to any great degree by temperature during storage after harvest with regards to flowering. The 7/8 cm size bulbs should be stored at 23°C from harvest in early summer until 1 September then at 30–35°C for 2 weeks followed by 5–9°C until planting. Smaller bulbs are stored at 18–20°C. Larger bulbs would not be used for propagation since they will readily flower after planting. Bulbs should be stored at 50–60% RH. Higher humidity will encourage the growth of *Penicillium*.

Commercial bulbs for forcing are handled in a number of different ways. After harvest, bulbs for very early forcing are stored at 30°C for a few days then exposed to 500 ppm ethylene for 24 h to stimulate floral initiation, especially in small bulbs or bulbs not exposed to high (>30°C) field temperatures. These bulbs should be planted for forcing as soon after ethylene treatment as possible. For later forcing, bulbs are stored at 30°C after harvest. Depending on cultivar, bulbs are stored at 5–9°C for 6–11 weeks prior to planting to stimulate flower development.

***LILIUM* (LILIES)** There are two basic types of lilies grown commercially: (i) the Easter lily (*Lilium longiflorum*); and (ii) *Lilium* species and hybrids. Easter lilies are usually forced in a greenhouse and grown as a potted flowering plant for the Easter holiday.

Production bulbs of Easter lilies are immediately replanted after harvest in late September. Commercial bulbs for potted plants are packed in moist peat moss after harvest. Bulbs can be handled in two different ways: (i) planted immediately and allowed to grow for 3 weeks at 15–16°C followed by 6 weeks at 2–7°C; or (ii) packed in moist peat moss and 'case cooled' at 2–7°C for 6 weeks then potted and grown in the greenhouse at 15–16°C. Once bulbs/plants have been chilled by either method, growth in the greenhouse is carefully monitored and flower development is regulated with temperature, light, water, fertility and chemical growth regulators so that blooming will coincide with the Easter holiday.

Hybrid and species lilies are used as fresh cut flowers, potted plants, or landscape specimens. Commercial bulbs are harvested, graded, cleaned and packed in moist peat moss. They are then wrapped in polyethylene for storage and shipping. Bulbs are cooled at 2°C for 6–8 weeks and then either planted for forcing or stored at between –1 and –2°C until needed for year-round forcing. Production bulbs are stored in moist peat moss at 2°C until planted.

***NARCISSUS* (HARDY DAFFODILS AND 'PAPERWHITES')** Two groups of *Narcissus* are used commercially: (i) hardy daffodils; and (ii) 'Paperwhite' *Narcissus*.

Production bulbs of daffodils are harvested, graded and then stored at 17–20°C until planting. For commercial production, bulbs are harvested, cleaned and graded. Bulbs for early forcing are given 1 week at 1.1°C followed by 17°C until planting. Bulbs for regular use are stored at 17–21°C.

'Paperwhites' are harvested, cleaned and graded and both production and commercial bulbs are then stored at 25–30°C under highly ventilated conditions until planting. If shoots emerge prior to planting, bulbs should be placed at 2°C to retard growth until desired for forcing. Prior to planting, bulbs need 2 weeks at a temperature between 9 and 17°C to ensure complete floral development.

***TULIPA* (TULIPS)** Tulips are the largest taxa of flowering bulbs grown worldwide, used as forced cut flowers, potted flowering plants, and landscape specimens. Bulbs are particularly sensitive to ethylene, so care in the entire harvest and storage chain must be taken to ensure that bulbs will not be exposed to it. Tulips are also susceptible to *Fusarium* and often acquire it in the field, thus routine inspections during storage are needed to remove infected bulbs. Additionally, decaying bulbs emit considerable ethylene which can lead to flower abortion and flowering abnormalities.

Production bulbs are harvested, cleaned and graded then stored at 23–25°C for 3–4 weeks. They are then stored at progressively lower temperatures

(23–20°C, then 17–15°C) until planting which enhances the production of large bulbs during propagation.

Commercial bulbs for forcing are given a 1 week exposure to 34°C immediately after harvest. They are then moved to a well-ventilated storage facility at 17–20°C. Once bulbs reach stage G (pistil formation) of floral development, they are moved to 5–9°C. If bulbs are moved before this stage is reached, flower abortion may occur. For later forcing, bulbs are stored at 17–23°C. Depending on the style of forcing, bulbs are given at least 8–10 weeks at 5°C prior to planting. When grown in temperate landscapes, bulbs that have been stored at 20–23°C are shipped and stored at 17°C until they are planted in the fall. They then receive their cold treatment naturally.

Herbaceous perennials

Herbaceous perennials often require storage at some point during their production chain. Most often, storage is between propagation and planting. The list of herbaceous perennials covers plants that are stored as dormant crowns with attached roots with no green leafy tissues attached (bare-roots) to those that are stored with fairly succulent leaves (greentops). In general, the less leafy material stored, the longer the storage life. There has been a considerable shift from storage of herbaceous plant material as overwintering bare-roots and greentops to storage as plugs. Plugs are small plants growing in plastic 30 cm × 60 cm trays that have from 32 to 516 cells.

Bare-roots and greentops

The biggest problems encountered during storage are desiccation, rot, and the growth of fragile buds once dormancy has broken. Desiccation can be prevented by storing propagules in poly bags. The polyethylene allows O_2 exchange while preventing moisture loss. Plants should be checked regularly to ensure that they are not drying out. Rots develop when propagules are not cleaned and appropriately dried before storage or when they enter storage infected with a rot-causing organism. Wood fibers or shredded newspaper added inside the poly bag absorbs excess moisture and condensation. Bud growth often occurs during shipping or retail storage. Every effort should be employed to maintain storage at –2°C until planting.

Propagules should be dormant before harvest. Species that do not have a dormant period should be harvested as late in the fall as possible. Mature plants or a late harvest help to ensure that the plants have maximum food reserves to survive storage. The major consequence of harvesting too early is a greatly reduced storage life.

After harvest propagules should be cooled to 0°C as quickly as possible to minimize respiration, which is often substantially increased due to wounding during harvest. As plants are processed for storage (cleaning, grading, washing, and packing) great care should be taken to avoid further injury, cool the product and to prevent anaerobiosis from occurring by maintaining adequate ventilation. Most propagules are stored at –2°C with shredded newspaper or some other dry filler inside unsealed poly bags which are placed in cardboard boxes (Cameron, 2004). Boxes should be appropriately sized to prevent overcrowding.

Some species such as *Gaillardia* and *Coreopsis* must be overwintered as potted plants rather than bare-root plants. The potted plants are placed inside poly liners to prevent desiccation, and then placed in cardboard boxes for storage. Some herbaceous perennials such as *Hibiscus, Alcea* and *Sidalcea* are chilling sensitive and can therefore only be stored at warm temperatures for a short time.

Plug plants

Plug production is the most widely utilized method of propagating herbaceous perennials. Either seeds or cuttings are planted in plugs of varying sizes and are ready for transplanting within 6–12 weeks. These plug trays are then sold to growers for transplanting into production containers for retail or wholesale markets. Plug plants are normally purchased and containerized in the fall and sold the following spring or summer.

Most herbaceous perennials require exposure to low temperatures to overcome dormancy to achieve acceptable regrowth in the spring. Containerized plants developed from plugs are often overwintered in minimally heated greenhouses to accumulate chilling. It is increasingly popular to supply a cold treatment to plants in the plug stage before planting. This reduces space requirements for chilling and provides more control over the exposure to the chilling temperature. Plugs can be stored at low temperatures to orchestrate production schedules with consumer demand. Plugs can be stored at 5°C

and held for 3 months with little damage as long as light levels high enough for maintenance photosynthesis is provided; the level of light required varies with species. Plugs stored in the dark for a long period do not normally survive transplanting.

Seeds

All seeds of commercially important horticultural crops must be stored for some quantity of time after harvest for food or as propagules for production (Walters and Towill, 2004). The major factor determining the storage life of viable seeds is moisture content and tolerance to desiccation. Three categories of seeds based on their desiccation tolerance are: (i) orthodox; (ii) recalcitrant; and (iii) intermediate.

Orthodox seeds are produced by most annual or biennial crops. They are extremely tolerant of nearly complete desiccation and are easily stored under cool, dry conditions for many years. Recalcitrant seeds are produced from herbaceous plants from aquatic habitats, tropical perennials, and some deciduous, temperate perennials. They are much less tolerant of desiccation and can be stored for a year or less at 92–98% RH. Tropical recalcitrant seeds are usually chilling sensitive and must be stored at >15°C. They can usually be stored from 2 weeks to 3 months. Temperate recalcitrant seeds are not chilling sensitive and are stored at 2–5°C and will survive storage for 6 months to 2 years. With all recalcitrant seeds, storage at high RH may lead to microbial contamination. High RH during storage often causes recalcitrant seeds to germinate. As soon as they do, they must be treated as seedlings and stored or planted appropriately. Intermediate seeds are produced by tropical and subtropical perennials and some nut species and can usually be stored for several years without a loss in viability. They are stored at 40–60% RH at 5°C and remain viable for 2–5 years under these conditions.

Seed maturity describes a stage of seed development where desiccation tolerance has been triggered in the seed and has fully developed. Immature seeds will not store well since the desiccation tolerance process has not been triggered or completed, thus any seed intended for storage should be mature at harvest. This is true for all three types of seeds. It is important to harvest orthodox seeds as soon as the seed is mature since exposure to high RH promotes seed deterioration.

High O_2 concentration in the storage atmosphere greatly reduces seed viability but the converse has not been demonstrated. Exposure to even low levels of light also decreases seed longevity therefore seed should always be dried and stored in the dark at 0°C. Storage at higher temperatures will reduce seed longevity by 50% for every 5°C increase in storage temperature.

Christmas trees

Cut Christmas trees are an important horticultural commodity in many parts of the world and their useful life is very dependent on postharvest practices (Hinesley and Chastagner, 2004). Cut trees may be shipped long distances and therefore must be stored for up to 1 month. Tree moisture greatly impacts longevity.

Once cut, Christmas trees begin to lose moisture. The moisture content of a tree is measured as water potential (symbolized as ψ) and is measured using a pressure bomb. A pressure bomb consists of a chamber in which a twig is inserted with just the cut surface protruding from the chamber. Pressure is applied to the chamber until moisture is observed at the cut. The force required to make moisture visible on the cut surface is reported as bars and the water potential is the negative value of this force. For example, if a sample requires 2 MPa (megapascals) of pressure to cause moisture to be visible at the cut surface, the water potential of that sample was –2, or $\psi = -2$ MPa (1 MPa is about –10 bars = –10 atmospheres). Drier samples require more pressure to produce moisture at the cut, indicating a lower (more negative) water potential.

If water potential is measured over time from when the tree is cut, two inflections can be observed in the time × pressure graph generated from the data. The first inflection point, V1, is observed soon after the tree first begins to dry out and varies with species and is normally –18 to –28 MPa. Once the tree reaches this first inflection point, the rate of drying slows down considerably. After a while, the tree begins to dry out more quickly and another inflection point is observed, V2. It appears that V2 corresponds to the moisture content where damage is first observed, such as needle dropping, discoloration, failure to rehydrate if placed in water. V2 ranges from –30 bars in eastern white pine (*Pinus strobus*) to –40 for Douglas fir (*Pseudotsuga menziesii*) and Fraser fir (*Abies fraseri*). During shipping and storage, the objective is to keep the

water potential above V2 to minimize damage upon display of the tree. Measuring V1 and V2 are valuable research tools, but are not routinely used by Christmas tree growers, brokers of the average consumer.

The main idea with all species is to never let the moisture content of a tree to reach a limit so low that it won't rehydrate if re-cut and placed in water. Since trees cannot be shipped in water, minimizing moisture loss between harvest and display are crucial. Once displayed, different species maintain and loose water at different rates. For example, noble fir (*Abies procera*) and Fraser fir (*A. fraseri*) maintain high moisture content for 4 weeks while eastern red cedar (*Juniperus virginiana*) and Atlantic white cedar (*Chamaecyparis thyoides*) only maintain high moisture content for 1 week before drying out, even when displayed in water.

A reasonable generalization is that a cut Christmas tree uses about 1 l of water/2.54 cm of trunk diameter/day, thus a moderate-sized tree will use 1 l/day. Many consumers do not use a large enough reservoir and allow the tree to dry out between watering. Once the tree has dried, it may not rehydrate very well, even if re-cut. Even though many additives have been suggested for prolonging tree longevity, the best solution to use for tree watering is plain water (Hinesley and Chastagner, 2004). Spray-on anti-transpirants do not extend tree longevity. The use of flame retardants is not recommended as they can injure needles. Maintain fire retardancy by keeping the tree moisture level as high as possible.

Trees that acclimate or become cold hardy last longer when cut compared with non-hardened trees for some unknown reason. The sugar raffinose increases in many trees during hardening but whether or not this influences tree longevity is not clear. Acclimated trees are also able to withstand exposure to cold between harvest and display, for example in the Christmas tree lot before it is sold. Non-acclimated trees that are exposed to cold after harvest suffer severe needle loss upon display, even if adequate water is supplied. Thus not only is species important when selecting a tree for longevity, but so is the environment from which it came.

17 Human Nutrition, Phytonutrients, Nutraceuticals and Horticulture

Plants are good for you. They contribute immensely to our mental well-being. Simply looking at a plant or a well-groomed landscape brings pleasure to anyone. The beauty surrounding us that is supplied by plants is amazing and is something we often take for granted. For those who are less than confident about their abilities or usefulness in this world, self-contentment from accomplishments derived from growing even a single plant from seed empowers individuals to believe in themselves again.

Plants keep us alive. Whether it is corn growing in Iowa or algae growing in the Sargasso Sea, plants generate the oxygen we breathe. Not only do they generate the oxygen we breathe, they provide us with all of our basic nutritional requirements for protein, fat, carbohydrate, fiber, vitamins, and minerals. Vegetarians and carnivores alike ultimately derive their nutrition from the same basic source: plants.

Over the last several decades, the study of nutraceuticals and phytonutrients has exploded as patients and doctors worldwide focus on the major key to good health: you are only as healthy as the food you eat. Many substances are found in plants that are important for protecting our bodies from stress, ageing, and disease. While many cultures have embraced this idea for centuries, much of the world is just beginning to grasp the concept.

This chapter will explore the contributions that plants make to our basic health and nutrition. A review of basic nutrition will be followed by a review of some of the latest work in the areas of phytonutrients and nutraceuticals. They are becoming increasingly importance as both preventive and therapeutic options in addressing human health.

All information presented in this chapter is for educational purposes only. No recommendations are endorsed or implied by the material presented herein. Please discuss your situation and any planned changes to your diet with a health care provider before embarking on any new diet plan.

Basic Nutrition

Basic human nutrition centers on a well-balanced diet that provides carbohydrates, protein, fat, fiber, vitamins, and minerals at levels that promote optimum health (Fig. 17.1). There is an overwhelming amount of literature available on the subject and just what balance of these key nutrients is appropriate remains a subject of great controversy. Even the recent guidelines jointly published by the USDA and US Department of Health and Human Services (2010) are subject to intense scrutiny (Hite *et al.*, 2010). Even though there are many opinions as to what constitutes an appropriate balance of nutrients for a particular definition of optimum health, some source must be used as a base reference. To that end, the 2010 recommendations of the USDA and US Department of Health and Human Services will serve as the basis for this discussion. What follows is a presentation of the most recent opinions of experts in the fields of nutrition and human health. What is best for you is your decision.

Their general recommendations can be summarized as follows:

1. Maintain an age and gender appropriate weight by balancing caloric intake and physical activity.
2. Reduce obesity by reducing caloric intake and increasing physical activity.
3. Limit sodium intake to 1500 mg/day.
4. Limit total daily caloric intake from saturated fatty acids to less than 10%/day.
5. Limit cholesterol consumption to less than 300 mg/day.
6. Avoid *trans* fats.
7. Avoid refined and processed foods.
8. Eat nutrient-dense foods such as dark-green, red and orange vegetables, whole grains, beans and peas, fruit, unsalted nuts and seeds, with small amounts of low or no fat dairy products, lean meat, poultry, and seafood.

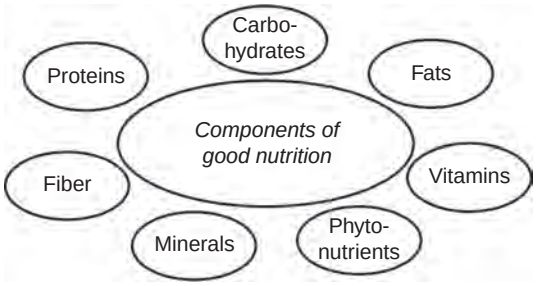


Fig. 17.1. The major components of good nutrition.

A major area of debate in nutrition literature surrounds the balance of carbohydrates, fats, and proteins in the diet that should be maintained for good health. The reader is left to investigate this aspect of nutrition and human health on their own.

Carbohydrates

The first major product of photosynthesis is a carbohydrate, glyceraldehyde 3-phosphate. From this compound, plants are able to synthesize a vast array of molecules in which the solar energy captured during photosynthesis can be stored for later use by the plant or used by another organism when it eats the plant. When humans consume plant-based foods, a major percentage of the energy derived from that food comes from carbohydrates. There are some exceptions where much of the stored energy is in the form of fat (avocado) or protein (soybeans).

Plant-based diets contain a myriad of distinct carbohydrates that impact human health differently (Englyst and Hudson, 1996). Categorization of carbohydrates occurs on three levels. The first is based on atomic composition and chemical bonds which distinguishes carbohydrates from fats, proteins, etc. The second level takes into account molecular configurations, chemical bonds, and physical properties. A third level addresses the nutritional properties and physiological responses to each species (Englyst and Hudson, 1996). The main nutritional properties of carbohydrates include whether or not they are hydrolyzed and absorbed in the small intestine and the relative glycemic response humans have to it. The glycemic index (GI) is a numeric scale from 1 to 100 that indicates how particular food impacts blood glucose levels (Porter, 2010). Foods with a low GI cause a slight to moderate rise in blood glucose soon after consumption while foods with a high GI cause a large and rapid rise in blood glucose levels that may be harmful for certain individuals, especially those with diabetes. A diet containing a large portion of carbohydrates with high GIs is generally considered unhealthy. Another aspect of carbohydrate impact on health is the negative effect high carbohydrate diets can have on blood triglyceride profiles and cardiovascular health risks (Acheson, 2010). There is increasing evidence that reducing caloric intake and reducing the amount of carbohydrates in the diet, replacing them with high quality proteins and unsaturated fats, leads to sustained weight loss and better blood lipid profiles from a cardiovascular disease risk standpoint (Acheson, 2010).

Carbohydrates are most often categorized to include simple sugars, sugar alcohols, starch, and non-starch polysaccharides (Fig. 17.2). Simple sugars include both monosaccharides and disaccharides. Monosaccharides are the true simple sugars, existing as single molecules that do not need to by broken down in the body before being absorbed into the bloodstream. The main monosaccharide consumed by humans is glucose (dextrose). It provides a quick source of energy, however, that energy is not sustained and once the ingested sugar is metabolized, a rapid drop in blood sugar occurs. In addition, glucose is converted to fatty acids and cholesterol in the liver then transported for deposition in adipose tissue (Vanderhoof, 1998). Other monosaccharides include among others, fructose (levulose), galactose, xylose, and ribose.

Disaccharides are sugars consisting of two molecules of the same or different monosaccharides joined together. Some common disaccharides include lactose (glucose + galactose), the only non-plant sugar in the human diet, sucrose (glucose + fructose), and maltose (glucose + glucose). Disaccharides must be broken down into their component monosaccharides before they can be absorbed into the bloodstream. This occurs fairly quickly after ingestion, particularly with sucrose and maltose, thus disaccharides also provide quick energy and may be followed by a large drop in blood sugar levels relatively soon after eating. Some individuals lack sufficient levels of the enzyme required for metabolizing lactose into glucose and galactose and suffer from lactose intolerance. In lactose-intolerant individuals, bacteria, rather than an enzyme, metabolize the lactose, producing uncomfortable amounts of gas and stomach acid in the small intestine.

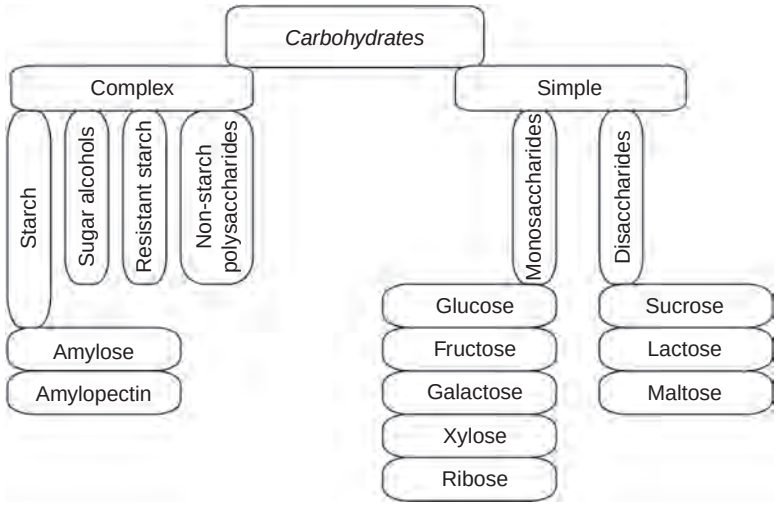


Fig. 17.2. The classes of carbohydrates found in a typical diet.

This can be prevented by avoiding foods high in lactose (dairy products) or by taking lactase (the enzyme that is lacking) supplements prior to consuming lactose-rich foods.

A sugar alcohol is a form of carbohydrate where the carbonyl group of a sugar has been hydrogenated forming a hydroxyl group, thus the classification as an alcohol. The simplest sugar alcohols, ethylene glycol and methanol are sweet tasting but toxic. The other sugar alcohols are generally sweet and non-toxic. Most sugar alcohols are consumed as food additives, not from ingestion of plant products containing sugar alcohols. Some of the more common sugar alcohols used as food additives include glycerol, erythritol, xylitol, mannitol, sorbitol, inositol, isomalt, and maltitol. They are not absorbed well and may be excreted in the urine (Englyst and Hudson, 1996). Sugar alcohols are not metabolized by oral bacteria, thus they do not promote tooth decay. When cooked, they do not caramelize. Many plants in the family *Rosaceae* produce significant amounts of sorbitol, celery (*Apium graveolens*) produces significant amounts of mannitol, and many seaweeds are rich in galactitol.

Polysaccharides are carbohydrates composed of a chain of many monosaccharide units. Starch (many molecules of glucose) is a very common polysaccharide. In many plants, glucose that is produced as a product of photosynthesis is often converted into starch for long-term storage in seeds, roots, stems, and fruit. When needed for energy or other metabolic processes, the starch is broken down into glucose. Since starch must be broken down before the glucose molecules can be used for energy production, starchy foods provide a longer, slower release of energy than either monosaccharides or disaccharides. Blood sugar levels are less likely to fluctuate wildly when a starch is consumed compared with simple sugars, thus consuming carbohydrates as starch is considered healthier than consuming monosaccharides or disaccharides.

The two main types of digestible starch in food are amylose and amylopectin. Amylopectin is more easily broken down than amylose, thus food containing high levels of amylopectin provides energy more rapidly than food containing high levels of amylose. The rate of digestion and energy release from starch also depends on how the starch is combined with other nutrients in the consumed food. For example, starch in whole foods such as whole grains are ingested with large amounts of fiber, thus the starch is more slowly digested compared with processed and refined foods where the fiber has been removed prior to consumption. The physical form of the starch also influences how digestible it is. Starch in a banana or a raw potato is present as granules that are resistant to degradation and digestion. When granular starch is cooked, it gelatinizes and is then readily digested.

Resistant starch is a polysaccharide that is resistant to digestion in the small intestine, and behaves more like fiber in the gastrointestinal tract. It may

be fermented in the large intestine by beneficial bacteria producing small-chain fatty acids and associated health benefits. Sources of resistant starch include brown rice (*Oryza sativa*), barley (*Hordeum vulgare*), whole wheat (*Triticum aestivum*), and buckwheat (*Fagopyrum esculentum*).

Dietary fiber

Dietary fiber has become an increasingly important component of good health. Nearly all of the dietary fibers we consume are plant-based carbohydrates (lignin is a polyphenol) that resist digestion in the small intestine. The characteristic of a carbohydrate that renders it digestible or non-digestible by humans is the nature of the bonds connecting adjacent sugar molecules. Amylose (starch) is a polymer of glucose molecules connected via alpha-1,4 glycosidic bonds and is digestible by humans. Change the bond between glucose molecules to beta-1,4 glycosidic bonds and we have cellulose which we cannot digest.

In order to have a comprehensive discussion concerning dietary fiber, a panel of experts gathered in 2001 to establish definitions for fiber that naturally occurs in plants (dietary fiber) and isolated fiber that might be used as a food additive or supplement (functional fiber) (Fig. 17.3). Total fiber is the sum of dietary and functional fiber and most adults need between 25 and 50 g fiber/day, depending on age and gender. While other classification systems for starch exist, this text will discuss fiber using the 2001 Institute of Medicine definitions and classification scheme (Institute of Medicine, 2002).

Dietary fiber includes lignin, cellulose, beta-glucans, hemicelluloses, pectins, gums, inulin and oligofructose, and resistant starch. Lignin is a complex polyphenol found in plant cell walls and seeds. Cellulose is a non-digestible (by humans) polymer of glucose found in plant cell walls. Beta-glucans are mixed glucose polymers with beta-1,4 and beta-1,3 glycosidic bonds. Oats and barley are particularly rich in beta-glucans. Hemicellusoses are polysaccharides with five- and six-carbon sugars, found especially in plant cell walls. Pectins and gums are viscous polysaccharides found primarily in fruits and seeds, respectively. Inulin is a mixture of fructose polymer chains that often terminate in a glucose molecule. Oligofructose is similar, except that its polymers are shorter and may terminate in either fructose or glucose. Plants that store inulin

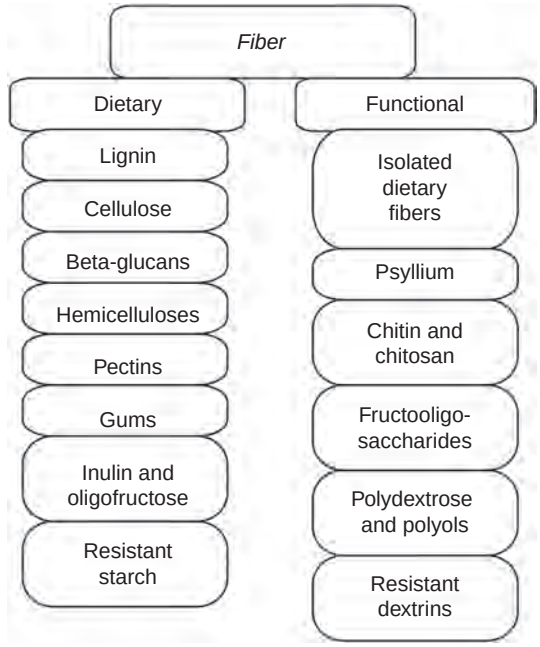


Fig. 17.3. Dietary and functional fibers in the typical human diet.

do not store starch. Some plants that have high levels of inulin include agave (*Agave* spp.), banana (*Musa* spp.), chicory (*Cichorium intybus*), dandelion (*Taraxacum officinale*), garlic (*Allium sativum*), Jerusalem artichoke (*Helianthus tuberosus*), jicama (*Pachyrhizus erosus*), and onion (*Allium cepa*). Inulins contain only 25–35% of the calories of starch and have a minimal effect on blood sugar levels, thus are potentially helpful in managing blood sugar-related illnesses. Resistant starch is starch that is isolated within plant cells and is inaccessible to digestive enzymes. Bananas and many legumes contain significant resistant starch.

Functional fiber is isolated, non-digestible carbohydrates that have either been extracted from plant material or manufactured. They benefit human physiology and are added to food or taken as a supplement (Institute of Medicine, 2002). Functional fiber includes isolated forms of dietary fiber, psyllium, chitin or chitosan, fructooligosaccharides, polydextrose and polyols, and reistant dextrins (Niness, 1999; Institute of Medicine, 2002; Hendler and Rorvik, 2008). Psyllium is a viscous mucilage extracted from the husks of psyllium (*Plantago ovata*) seeds. Chitin is a non-digestible carbohydrate isolated from the shells of crustaceans

such as crabs and lobsters. Chitin is a long polymer of acetylated glucosamine units. When chitin is deacetylated it becomes chitosan. Fructooligosaccharides are food additives composed of synthetic fructose polymers terminating in a glucose molecule. Polydextrose and polyols are synthetic polysaccharides added to processed foods for bulk or as a sugar substitute. Resistant dextrins (also called resistant maltodextrins) are indigestible polysaccharides synthesized by heating starch with certain enzymes. They are used as food additives.

Other descriptions of fibers include: (i) viscous versus non-viscous; (ii) fermentable versus non-fermentable; and (iii) soluble versus insoluble. Viscous fiber is one that will form a viscous solution or gel with water to produce bulk which tends to delay emptying of the stomach (Lupton and Turner, 2000; Gallaher and Schneeman, 2001). Viscous fibers include pectins, beta-glucans, some gums, and mucilages (psyllium). Fibers may also be categorized as fermentable or non-fermentable by bacteria in the gut. Fermentation products often include gas and short-chain fatty acids, which can be used as an energy source and may help prevent cardiovascular disease. Many fruits and vegetables are high in fermentable fiber. Fiber rich in cellulose is not fermentable. Terms describing the solubility of fiber in water (soluble versus insoluble) were originally used to simply describe this chemical property. Over time, soluble fiber was used as a term describing the potential for bacterial fermentation and thus health benefits, even though the association is somewhat misleading (Marlett, 1992). Other fibers beside soluble fibers have health benefits. When considering fiber as beneficial to health, specific fibers should be discussed.

Increasing viscous fiber intake can significantly lower total serum and low-density lipoprotein (LDL) cholesterol (Brown *et al*., 1999) and improve blood sugar control (Wolever and Jenkins, 2001) preventing postprandial spikes in blood glucose levels. Both of these factors appear to play a role in the reduced risk of cardiovascular disease associated with increased fiber intake (Liu and Willett, 2002). Increasing any form of dietary fiber improves intestinal flow and relieves constipation by softening the stool and increasing the rate of passage through the intestine. Enhanced regularity may contribute to the reduced risk for colon cancer associated with increased fiber consumption (Bingham *et al*., 2003) by quickly eliminating potential carcinogens from the body. While increased fiber intake has long been associated with a reduced risk of colorectal cancer, a number of controlled studies have failed to establish causality between the two (Alberts *et al*., 2000; Bonithon-Kopp *et al*., 2000; Schatzkin *et al*., 2000; Ishikawa *et al*., 2005). The reasons for such a discrepancy are unknown. High fiber intake coupled with a low fat diet seems to reduce the risk of breast cancer (Dong *et al*., 2011). Increased fiber consumption extends satiety after a meal, thus those with higher fiber intake tend to weigh less than those consuming less fiber (Wanders *et al*., 2011).

Some of the best sources of dietary fiber include legumes, oats, wheat and rice bran, and most fruits and vegetables. Sources for functional fibers vary depending on fiber type. Beta-glucans are generally extracted from oats, barley, mushrooms, yeast, and algae (Hendler and Rorvik, 2001). Pectin is derived from citrus rinds and apple pulp. Inulins and oligofructans are synthesized from sucrose or extracted from chicory root and are often called prebiotics since they have the capacity to stimulate the growth of beneficial bacteria (*Bifidobacteria*) in the intestine (Gibson *et al*., 1995). Guar gum is taken from the Indian cluster bean and psyllium is isolated from psyllium husks. Chitosan is derived from chitin obtained from shells of crustaceans.

Protein

Proteins constitute the main structural components of the human body. Besides their structural importance, enzymes are proteins, and enzymes are responsible for orchestrating nearly all metabolic activity in any living organisms. They are also important for regulation of cellular import and export as they form channels in cell membranes through which entry into and exit from the cell is carefully controlled.

The human body is constantly synthesizing new proteins to replace those lost to injury or senescence, or those needed for maintenance and growth. In order to manufacture needed proteins, the human body requires amino acids, basic building blocks that are connected to form proteins. There are 20 amino acids available from which to construct proteins. The number, species and arrangement of the amino acids in a protein give that protein its unique structure and function. Proteins in general can be from 51 (the hormone insulin) to 3000 (the ATPase complex in the mitochondria) amino acids in length.

When we eat proteins they are digested and broken down into amino acids before being absorbed into the bloodstream to be transported to cells throughout the body. In general, protein molecules are larger than carbohydrate molecules and thus take longer to digest. As such, proteins provide a longer, slower source of energy compared with carbohydrates. In general, humans require between 40 and 65 g of protein/day to survive, about 0.8 g/kg of body weight (Porter, 2010). If this amount of protein is not ingested, the body will begin to attack and break down its own muscles. If too much protein is ingested, it will be broken down by the body and stored as fat.

While there are 20 amino acids needed by the human body for survival, only 11 of them can be synthesized from molecules in our bodies. The other nine amino acids, called essential amino acids, must come from the food we eat. We all need eight of the essential amino acids: (i) isoleucine; (ii) leucine; (iii) lysine; (iv) methionine; (v) phenylalanine; (vi) threonine; (vii) tryptophan; and (viii) valine (Fig. 17.4). Infants need the ninth one, histidine (Porter, 2010). While nearly all of the protein we eat has most of the 20 amino acids in them, the proportion of specific amino acids is important for good health. Additionally, protein from different sources varies in terms of how well our bodies can utilize the amino acids in them. Protein derived from animals has a balance of the different amino acids very similar to our own tissues, thus animal-derived protein is often called complete. Many plant-derived proteins lack or are low in one or more of the essential amino acids, thus many consider plant proteins incomplete or of lower quality. However, different plant-based foods have different amino acid profiles, so if the plant-based proteins from various sources are correctly combined, the protein profile is complete.

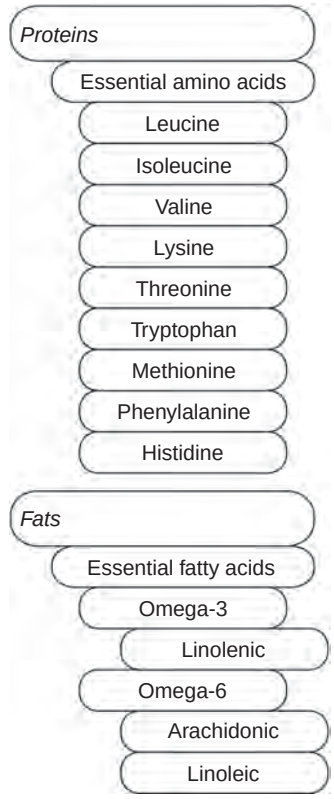


Fig. 17.4. The essential amino acids and fatty acids we cannot synthesize. They must be obtained from the foods we eat.

Fat

Fats are energy-dense molecules composed of glycerol with attached fatty acids. Our bodies can synthesize many of the fatty acids it needs. Others, however, cannot be synthesized and must be obtained from the food we eat. They are called essential fatty acids (Fig. 17.4). In general, essential fatty acids are about 7% of the fat consumed each day (Porter, 2010). The essential fatty acids are: (i) alpha-linolenic acid (an omega-3 fatty acid); and (ii) linoleic acid (an omega-6 fatty acid). Good sources of omega-3 fatty acids include flaxseed, lake trout, mackerel, salmon, herring, tuna, green leafy vegetables, and walnuts. Omega-6 fatty acids can be found in vegetable oils such as sunflower, safflower, corn, cottonseed, and soybean oils, fish oils and egg yolks (Porter, 2010). Omega-3 fatty acids may reduce the risk of coronary artery disease.

While fats are often perceived very negatively, they are required for growth, energy, and overall good health. The type(s) of fatty acid attached to the glycerol molecule determines whether the fat is considered a 'good' fat or a 'bad' fat from a nutrition point of view. The double bonds within a fatty acid may be saturated with hydrogen atoms, and are hence called saturated fatty acids. If available double bonds are not saturated, the fatty acids are called unsaturated fatty acids. The degree of fatty acid saturation is important in determining the health benefits or lack thereof for the fats we consume. Much of the fat from animal sources is saturated while most plant sources contain unsaturated fats.

The exceptions are palm and coconut oil, both are highly saturated. *Trans* fats are completely man-made by hydrogenating unsaturated fats, often vegetable oils. Saturated and *trans* fats are associated with increased blood cholesterol levels and increased risk of heart disease, thus we are encouraged to minimize the amount of saturated and *trans* fat we consume each day.

A general recommendation for fat consumption is to limit it to less than 30% of the daily caloric needs (less than about 90 g of fat/day) and limiting the amount of saturated fat to less than 10% (less than 30 g/day). In order to get our essential fatty acids, foods rich in both omega-3 and omega-6 fatty acids should replace saturated and *trans* fats in our diets.

Vitamins

Vitamins are organic compounds required by humans in small amounts that are essential for metabolism. They cannot be synthesized by humans, thus they must be obtained in the diet. In order for an organic substance to be considered a vitamin, a lack of it in the diet must produce clear and unmistakable symptoms of deficiency.

Vitamins are either water soluble (vitamin C and the vitamin B complex, which includes biotin, folate (folic acid), niacin, pantothenic acid, riboflavin (vitamin B2), thiamine (vitamin B1), vitamins B6 (pyridoxine) and B12 (cobalamins)) or fat soluble (vitamins A, D, E, and K) (Porter, 2010) (Fig. 17.5). All fat-soluble vitamins and the water-soluble vitamin B12 are stored in the liver and fatty tissue. Low fat diets might lead to a deficiency of these vitamins, and health disorders that interfere with fat absorption such as Crohn's disease, cystic fibrosis, and pancreatitis may also lead to a deficiency. Water-soluble vitamins are not stored in the body and are often eliminated in urine.

Many factors determine whether or not vitamins are lost during cooking. In general, brief to moderate cooking does not destroy fat-soluble vitamins (A, D, E, and K) and cooking foods with water-soluble vitamins tends to leach the vitamins into the cooking liquid. If the liquid is discarded rather than consumed, vitamins are discarded as well. Factors such as cooking temperature, length of cooking, light exposure, and pH all affect the stability of vitamins, thus making a blanket statement about the stability of most vitamins during cooking is impossible. There are a few observations about stability that can be made for some vitamins.

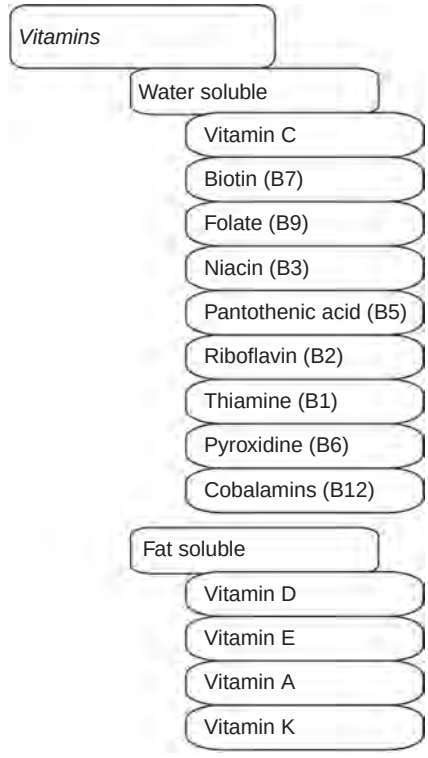


Fig. 17.5. Vitamins required for good human health.

Vitamin C is easily destroyed by heat, but is more heat stable under acidic conditions, such as in the heat pasteurization of orange juice (Morris *et al.*, 2004a). Loss of vitamin C due to oxidation is accelerated by copper or iron, thus using cast-iron or copper cookware may accelerate vitamin C loss. Riboflavin is moderately heat stable under neutral pH; however, it is easily destroyed under alkaline conditions (Morris *et al.*, 2004a). Riboflavin is also destroyed by light at neutral and alkaline pHs. Niacin is relatively heat stable while thiamine is destroyed by heat (Morris *et al.*, 2004a). Folate is destroyed by prolonged heating or by food preparation with copper utensils. Vitamin B6 is fairly heat stabile under alkaline or acidic conditions, however, the pyridoxal form of the vitamin is heat labile (Morris *et al.*, 2004a). Vitamins A and D are destroyed by heat.

Biotin (B7)

Biotin is a non-toxic, heat-stable, water-soluble vitamin that is attached to the active site of

carboxylase enzymes (Higdon *et al*., 2012). Carboxylase enzymes are particularly important in fatty acid metabolism, gluconeogenesis (the production of glucose from protein or fat), and leucine catabolism. Biotin also plays a role in DNA replication and transcription.

Biotin deficiency is rare but may occur in patients that have been fed intravenously without biotin supplementation. Deficiency may also occur in individuals who have consumed raw egg whites for a prolonged period, as biotin binds to a protein in raw egg whites called avidin which prevents biotin absorption.

Since there is not enough scientific data available to establish a recommended dietary allowance (RDA) for biotin, an adequate intake (AI) has been established (Higdon *et al*., 2012). It ranges from 5 μg/day for infants to 8–20 μg/day for children, the amount increasing with age, 25 μg/day for adolescents, and 30 μg/day for adults. Women who are breastfeeding have an AI of 35 μg/day.

Good sources of biotin include yeast, egg yolks, and liver. The best plant-based source of biotin is Swiss chard (*Beta vulgaris* subsp. *cicla*), containing about 10 μg biotin per one cup serving. Intestinal bacteria synthesize biotin and this biotin may be absorbed by the body providing another source of the vitamin.

Folic acid (B9)

Folic acid, a water-soluble B vitamin, rarely occurs in the human body or in foods (Higdon *et al*., 2012) but is the form of this B vitamin normally found in supplements. Folates are the forms found in food or the human body and they come in many chemical configurations. Folates are non-toxic, however, ingestion of large doses of folic acid may mask a B12 deficiency which may cause serious neurologic damage. Folates are critical enzyme cofactors important in nucleic acid and amino acid metabolism. DNA synthesis depends on folate coenzymes and the synthesis of methionine also depends on folates. Methionine is need for the production of *S*-adenosyl-L-methionine (SAM) which is important in many methylation reactions, including methylation of DNA. Methylation of DNA may help prevent cancer. Folates are also important in the synthesis of methionine from homocysteine and a deficiency of folate may lead to a build up of homocysteine which has been implicated as a risk factor for heart disease.

Folate may be deficient due to a dietary insufficiency or it may be induced by alcohol consumption, pregnancy, cancer, or when large amounts of non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin or ibuprofen, are taken in large therapeutic dosages. Folates are particularly important in the development of the nervous system of a fetus, and deficiency can lead to neural tube birth defects. Nervous system development occurs during the first month of pregnancy. Since many women do not know they are pregnant during the first month, folate nutrition is particularly important in women of child-bearing age.

The RDA for folate is most often reported as micrograms of dietary folate equivalents (DFE), which reflects greater availability of synthetic folic acid found in dietary supplements and fortified foods compared with naturally occurring folates in food. One microgram of folate from food provides 1 μg of DFE, while 1 μg folic acid taken with food provides 1.7 μg DFE, and when taken on an empty stomach, provides 2.0 μg DFE (Higdon *et al*., 2012). Infants require 65–80 μg folate (actual folate, not as DFE), the requirement increasing with time. Children require 150–300 μg DFE, increasing with time, adolescents and adults 400 μg DFE. Women who are pregnant or might become pregnant have an RDA of 600 μg DFE and those breastfeeding, 500 μg DFE (Higdon *et al*., 2012).

Green leafy vegetables are an excellent source of folates (hence the name, folate). Other plant-based foods rich in folate include lentils, garbanzo beans, lima beans, pinto beans, black beans, kidney beans, orange juice, and asparagus. Prolonged cooking can reduce the folate content of foods significantly.

Niacin (B3)

Niacin, also known as vitamin B3 or nicotinic acid, is a water-soluble vitamin important in forming the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), both extremely important in energy metabolism (Higdon *et al*., 2012). Both NAD and NADP are important in cellular oxidation–reduction reactions, processes involving electron transfer during metabolism. NAD and NADP function as electron donors or acceptors in many cellular reactions. NAD is normally involved in catabolism of compounds while NADP is usually involved in anabolic reactions (biosynthesis of compounds). NAD is also very important in cell signaling, transcription,

apoptosis, DNA repair, and stress responses. Considering these roles of NAD, niacin may be important for cancer prevention.

Niacin deficiency caused the problem known as pellagra in Europe in the 1700s and in the southern USA in the 1900s. The common theme of both locations and times was the widespread consumption of corn and its products as the main dietary staple, especially among the less advantaged. While corn contains significant amounts of niacin, it is relatively unavailable. Cooking corn in an alkaline solution releases the niacin making it available. Even though a considerable amount of corn is consumed as the main dietary staple in Mexico, pellagra is rare. This is because the corn is soaked in a calcium oxide solution prior to cooking, greatly increasing niacin's bioavailability.

Niacin is either consumed in food or formed in the liver from the amino acid tryptophan. Approximately 60 mg of tryptophan are required to synthesize 1 mg niacin. Dietary niacin is reported as niacin equivalents (NE) which represents the ingestion of either 1 mg niacin or 60 mg tryptophan. The RDA for niacin is 2–4 mg NE/day for infants, 6–12 mg/day for children, and 14–16 mg/day for adults. Women who are pregnant should consume 18 mg NE/day (Higdon *et al*., 2012).

Good sources of niacin include yeast, meat, poultry, and red fishes. Good plant sources of niacin include legumes and seeds, and to a lesser degree green leafy vegetables, coffee, and tea. In some plant products the niacin is bound to a carbohydrate, greatly reducing its bioavailability. Niacin is not easily destroyed by heat, however, it is water soluble, thus when foods are cooked in a liquid, the liquid and the food should both be consumed.

Pantothenic acid (B5)

Pantothenic acid (vitamin B5) is found in all cells as a component of coenzyme A (CoA), vital for all life (Higdon *et al*., 2012). CoA is important in energy metabolism, in the synthesis of essential fats, cholesterol, steroid hormones, acetylcholine, hemoglobin, and melatonin. Much of the metabolism occurring in the liver requires CoA.

Pantothenic acid deficiency is very rare. There is no RDA for pantothenic acid, however, the AI often recommended ranges from 2 mg/day in infants to 5 mg/day in adults, 6 mg/day for women who are pregnant and 7 mg/day for women who are breastfeeding.

Good sources of pantothenic acid are yeast, egg yolks, yogurt, milk, liver, and kidney. Good plant sources include broccoli, legumes, mushrooms, avocado, sweet potatoes, and non-processed whole grains. Processing, freezing or canning of food products may result in a 35–75% loss of pantothenic acid (Food and Nutrition Board, Institute of Medicine, 1998).

Riboflavin (B2)

Riboflavin, also known as vitamin B2, is a water-soluble vitamin important as part of the coenzymes flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) (Food and Nutrition Board, Institute of Medicine, 1998). These coenzymes are important in energy metabolism and the metabolism of drugs and toxins. FAD is an integral component of the electron transport chain in mitochondria. Both coenzymes are also important in the function of a number of antioxidant enzymes such as glutathione reductase and glutathione peroxidase. The production of uric acid, a potent antioxidant in the blood, requires FAD. Riboflavin is also important in the metabolism of many of the other B vitamins and iron absorption (Higdon *et al*., 2012).

Riboflavin deficiency by itself is rare. However, it may occur concomitantly with deficiencies of other water-soluble vitamins. The RDA is around 1 mg/day, slightly less for infants and children and slightly more for adult males, and women who are pregnant or breastfeeding. Nearly all food contains some riboflavin. Some plant sources particularly rich in riboflavin are almonds, broccoli, asparagus, and spinach. Riboflavin is particularly sensitive to light degradation.

Thiamine (B1)

Thiamine (thiamin) is a water-soluble vitamin also known as B1. It was one of the first substances identified as a vitamin (Higdon *et al*., 2012). It occurs in humans either as free thiamine or in its phosphorylated form. Thiamine pyrophosphate (thiamine with two phosphate groups attached) is an important coenzyme for several life-essential enzyme systems, particularly enzyme systems involved in energy generation from food and in the synthesis of nucleic acids.

Severe thiamine deficiency leads to beriberi which affects the nervous, muscular, gastrointestinal, and cardiovascular systems. Dry beriberi is characterized by peripheral neuropathy, a tingling,

burning or numb sensation in the feet or hands. Muscle pain and seizures may also occur. Wet beriberi leads to rapid heart rate, an enlarged heart, severe edema and eventually congestive heart failure. Cerebral beriberi leads to abnormal eye movements, an odd gait, and confused memory.

Thiamine deficiency may be the result of inadequate intake, alcoholism, an increased thiamine requirement brought on by strenuous physical activity, pregnancy or growth spurts during adolescence, excessive loss from the body due to excessive urination, or consumption of anti-thiamine factors in food (Higdon *et al*., 2012). Anti-thiamine factors are substances present in some foods (coffee, tea) that create an oxidized, inactive form of thiamine. Thiaminases are enzymes, normally destroyed during cooking, that are present in certain raw foods (freshwater fish, shellfish, and ferns) that break down thiamine. Individuals who consume large quantities of these raw thiaminase-containing foods could suffer a thiamine deficiency.

The RDA for thiamine ranges from 0.2 mg/day for infants to just over 1 mg/day for adults. Pregnant women or women who are breastfeeding require around 1.4 mg/day. Good plant sources of thiamine include whole grains, beans and lentils, nuts, spinach, orange, and cantaloupe. Much of the thiamine is lost during processing of white flour and polished rice, and products made with these products.

Vitamin A

Vitamin A is not a single substance, but rather a general term for a large number of related compounds. Major compounds in this class include: (i) retinol (an alcohol); (ii) retinal (an aldehyde); and (iii) retinoic acid, an irreversibly oxidized form of retinol. All are known as retinoids (Higdon *et al*., 2012). Dietary recommendations are based on the amount of retinol or the retinol equivalents from other forms that are available from foods. Beta-carotene is a provitamin A carotenoid which can be converted into retinol. Plants produce many different carotenoids, however, only a limited number can be converted into retinol.

Retinol is very important for night vision as it is part of the pigment rhodopsin located in the rod cells of the retina. Rod cells are capable of detecting extremely low levels of light. When rhodopsin is stimulated by a photon of light, a series of biochemical reactions occurs leading to the generation of an electrical impulse which ultimately is converted by the brain into vision. Different forms of retinoic acid act as hormones that affect the expression of genes responsible for cellular differentiation and many of the responses to retinol that are observed are results of this regulation. Vitamin A is also important for proper immune system function, specifically by maintaining the integrity and function of mucosal and skin cells (Higdon *et al*., 2012). Retinol and retinoic acid are crucial for proper differentiation and development of white blood cells important in immune responses. Differentiation of red blood cells and the release of iron from cellular storage sites for incorporation into hemoglobin are both regulated by retinoids. Vitamin A is also important for normal heart, eye and ear development in the fetus.

The RDA for vitamin A is reported as micrograms of retinol activity equivalents (RAE)/day. Different forms of vitamin A and provitamin A carotenoids have different potencies when expressed as equivalents to retinol, thus the dietary value of foods and daily requirements for vitamin A are often expressed as RAEs. For example, 12 μg of beta-carotene from food provide the equivalent dietary benefit to 1 μg retinol, thus 1 μg of beta-carotene from food has the potency of 0.0833 RAE. (Beta-carotene in oil as a supplement has an RAE of 0.5.) The older vitamin A standard, the international unit (IU) provides 0.3 μg RAE. Daily RAE requirements range from 400 to 900 μg RAE/day depending on age. Women who are pregnant or breastfeeding require significantly more vitamin A, 1200 and 1300 μg RAE/day, respectively. It is important to note that therapeutic doses of retinol have been shown to cause birth defects, thus great care must be taken when using any vitamin A supplement or product containing vitamin A.

Retinol in its free form is not normally found in foods. A storage form of retinol, retinyl palmitate, is often found in animal-derived foods. Plant-based foods contain many carotenoids but only four (beta-carotene, alpha-carotene, gamma-carotene, and the xanthophyll beta-cryptoxanthin) can be converted into vitamin A by the body. Cod liver oil is particularly rich in retinol (1350 μg RAE per teaspoon). Good plant sources of 'vitamin A' include carrots, sweet potato, pumpkin, cantaloupe, mango, spinach, kale, collards, and butternut squash. Since vitamin A and its precursors are fat soluble, maximum nutritional benefit is obtained by consuming cooked vegetables with a little bit of fat or oil.

Vitamin B6

Vitamin B6 is a water-soluble vitamin that exists in three forms: (i) pyridoxal (PL); (ii) pyridoxine (PN); and (iii) pyridoxamine (PM) (Higdon *et al*., 2012). The most nutritionally important form of vitamin B6 is the coenzyme pyridoxal 5′-phosphate (PLP). Humans cannot synthesize vitamin B6. PLP is particularly important in the release of glucose from glycogen in muscle tissue and for the synthesis of glucose from amino acids (gluconeogenesis). In the nervous system, synthesis of neurotransmitters such as serotonin, dopamine, norepinephrine, and gamma-aminobutyric acid requires PLP. PLP is also required for: (i) the production of the iron-containing portion of hemoglobin; (ii) the conversion of tryptophan into niacin; and (iii) the synthesis of nucleic acids. PLP regulates the function of steroid hormones such as testosterone and estrogen by binding to receptor cites, thus decreasing their effect.

Daily vitamin B6 requirement increases with protein intake. The RDA for vitamin B6 ranges from 0.1 mg to 2.0 mg/day, depending on age and sex. Salmon, chicken, and turkey are fairly high in vitamin B6. Plant-based foods particularly high in vitamin B6 include bananas, baked russet potato, spinach, and hazelnuts.

Vitamin B12

Vitamin B12 has the most complex structure and is the largest in size of the vitamins (Higdon *et al*., 2012). It is unique in that it contains the metal cobalt, and the term 'cobalamin' is used to describe compounds having vitamin B12 activity. Methylcobalamin and 5-deoxyadenosyl cobalamin are two forms of vitamin B12 used by the human body. Methylcobalamin is important for the proper functioning of the folate-requiring enzyme that converts homocysteine into methionine, which is converted into SAM which donates a methyl group during methylation of DNA. Methylation of DNA has been associated with cancer prevention. Homocysteine accumulation, which would occur if vitamin B12 or folate were deficient, has been linked to increased risk for heart disease. Vitamin B12 is also needed for energy production from fats and proteins and for hemoglobin synthesis.

Uptake of vitamin B12 is somewhat complicated. After being released from foods by acids and enzymes in the stomach, vitamin B12 binds to proteins called 'R' proteins. After passing into the small intestine, vitamin B12 is released from the R proteins and binds to a protein called the 'intrinsic factor' (IF). If calcium supplied by the pancreas is present, the IF–B12 complex is absorbed by the small intestine. If the stomach, pancreas or small intestines are not functioning well, vitamin B12 must be absorbed passively, a very inefficient process.

The RDA for vitamin B12 ranges from 0.4 μg to 2.4 μg/day, depending on age, with pregnant or nursing mothers requiring slightly more. Older individuals are encouraged to get their vitamin B12 from fortified foods or supplements since they may suffer from a reduced digestive capacity and vitamin B12 may remain unavailable for absorption. Vitamin B12 is generally not present in plant-based foods, thus all dietary requirements must be met through animal-based foods such as fish, seafood, meat, or dairy, or from supplements.

Vitamin C

Vitamin C (ascorbic acid) is a water-soluble vitamin that cannot be synthesized by humans (Higdon *et al*., 2012). Vitamin C is required for: (i) synthesis of the structural component collagen; (ii) the neurotransmitter norepinephrine; and (iii) the synthesis of carnitine, a molecule essential for transporting fat into mitochondria for energy production. Vitamin C is also a powerful antioxidant. The sometimes fatal disease known as scurvy is due to a vitamin C deficiency and is characterized by bruising, bleeding, tooth and hair loss as well as lack of energy. In most cultures, scurvy is rare since only 10 mg of vitamin C daily will prevent it and most humans generally consume significantly more than that each day.

The RDA for vitamin C ranges from 40 to 90 mg/day for infants and adults, respectively. Smokers are encouraged to consume an additional 35 mg/day to combat the oxidative stress from smoking toxins. Woman who are pregnant or who are breastfeeding require around 120 mg/day. Many plant-based foods are high in vitamin C including: citrus fruits, strawberries, sweet red pepper, and broccoli.

Vitamin D

Vitamin D, a fat-soluble vitamin, is needed for normal calcium metabolism (Higdon *et al*., 2012). Upon skin exposure to UV B radiation in sunlight, humans can synthesize vitamin D3 (cholecalciferol).

Yeasts, higher fungi, and some higher plants can synthesize ergosterol which can be converted to vitamin D2 (ergocalciferol) by UV light (Holick *et al*., 2002). The generic term 'vitamin D' normally refers to either or both vitamin D3 and D2. In order to be metabolically useful, vitamin D must be converted to 25-hydroxyvitamin D (calcidiol) in the liver followed by conversion to 1,25-dihydroxyvitamin D (the most potent form of vitamin D) in the kidney. The physiological activity of vitamin D is due to its regulation of nuclear gene transcription.

Vitamin D has a number or roles including: (i) being integral in balancing calcium levels in the blood; (ii) reducing cell proliferation while inducing differentiation; (iii) it may enhance the immune system; (iv) it helps regulate insulin secretion by the pancreas especially in type 2 diabetics; and (v) it may help decrease the risk of hypertension. A deficiency of vitamin D may lead to rickets, bowing of weight-bearing limbs, especially in infants and children. It may also lead to bone weakness and softening in older individuals as well as muscle weakness and pain. The RDA for vitamin D increases from 10 μg/day in infants to 15 μg/day in adults and 20 μg/day in older adults (>70 years). Most people can get their vitamin D requirement from 5–10 min of sun exposure each day. Dark-skinned individuals and those who use copious sunscreen may require dietary vitamin D. Additionally individuals living above latitudes 40°N or 40°S may require vitamin D supplementation during the winter as insufficient UV radiation occurs during these months. Most foods contain very little vitamin D unless they are fortified, thus supplements may be required if adequate sun exposure is not available.

Vitamin E

While there are eight antioxidants that belong to the vitamin E family, only alpha-tocopherol is maintained at appreciable levels in the human body (Higdon *et al*., 2012). Blood serum gamma-tocopherol levels are much lower than alpha-tocopherol levels, even though much of our food contains gamma-tocopherol. The main function of vitamin E is to remove free radicals from the human body, especially protecting fat molecules in membranes and LDLs from oxidation. Oxidized membrane lipids lead to loss of membrane integrity while oxidized LDLs are implicated in cardiovascular disease. Even though the antioxidant capacity of an alpha-tocopherol molecule is lost once it has been oxidized by a free radical, the antioxidant capacity can be regenerated by other antioxidants such as vitamin C. Vitamin E is also important in cell signaling, immune system function, blood platelet aggregation, and vasodilation.

RDA levels for vitamin E range from 4 mg/day in infants to 15 mg/day in adults and are generally expressed as milligrams of alpha-tocopherol. Women who are breastfeeding need around 19 mg/day. Good sources of vitamin E include olive, corn, canola, soy, safflower, and sunflower oils, almonds, hazelnuts, and peanuts.

Vitamin K

Vitamin K is a fat-soluble vitamin vital for blood coagulation (Higdon *et al*., 2012). The two forms of vitamin K are K1 (phylloquinone), the predominant form in our diet, produced by plants and vitamin K2 (many forms of menaquinones) which is produced by bacteria in the intestines of animals. Vitamin K is also important in the production and activity of 'Gas6', a protein that seems to be involved in cell signaling, proliferation, and adhesion, and also appears to have anti-apoptosis properties.

There is no RDA for vitamin K. However, there is a recommended AI which is around 2 μg/day for infants, 30 μg/day for children 1–3 years, 55 μg for children 4–8 years, 60 μg for children 9–13 years, 75 μg/day for adolescents, and 120 μg/day for adult males and 90 μg/day for adult females. Pregnant or breastfeeding women who are 18 years of age or younger should reduce their vitamin K intake to 75 μg/day. Vitamin K1 is the predominant form of dietary vitamin K and foods rich in vitamin K include kale, broccoli, Swiss chard, and parsley.

Minerals

Minerals are elements that living organisms cannot synthesize. They are obtained from the earth mostly by bacteria, fungi, or plants. Most minerals in our diet are derived from plants. We either eat the plant directly, or consume animals (or their products) that have eaten the plants. Some minerals are also obtained through the water we drink. Since soils and water sources vary around the world, so do the levels of minerals in plants, animals, and water.

There are two classes of minerals: (i) macrominerals; and (ii) microminerals (Fig. 17.6). Macrominerals are those minerals we require in relatively large quantities for good health. They include calcium, chloride, magnesium, phosphorus, potassium, and sodium. Microminerals are no less important, however, they are required in much lower amounts. Microminerals include chromium, copper, fluoride, iodine, iron, manganese, molybdenum, selenium, and zinc (Porter, 2010). Trace minerals such as arsenic, cobalt, fluoride, nickel, silicon, and vanadium which seem to be essential in animal nutrition have not been established as essential in human nutrition.

This section reviews the macrominerals and microminerals essential to human health, their general functions in the human body, and good dietary sources of each.

Calcium

The most common mineral in the human body is calcium and most of it is found in teeth and bones (Higdon *et al*., 2012). While less than 1% of the calcium found in the body is in the blood and extracellular fluid, the levels in these fluids are critical for good health. The major component of our skeletal system and our teeth is calcium and phosphate-rich hydroxyapatite.

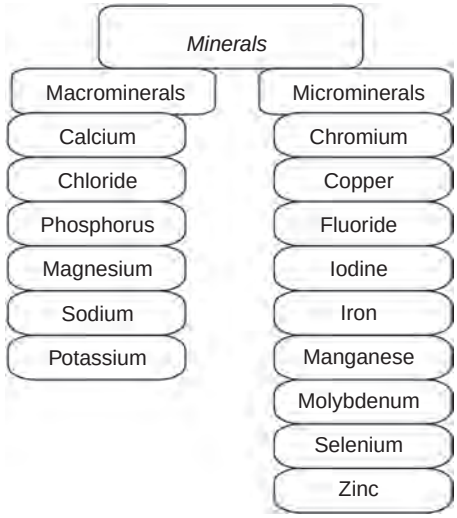


Fig. 17.6. The essential minerals we need to maintain good health.

We usually don't think of it, but our bones are in a constant state of resorption and synthesis throughout our lives. Our bodies resorb bones while specialized cells called osteoblasts regenerate new bone. Calcium is also extremely important in cell signaling for important bodily functions such as vasoconstriction and vasodilation, nerve impulse transmission, muscle contraction, and insulin secretion. Calcium is also an important cofactor for enzyme and protein function. Blood and extracellular fluid calcium levels are highly regulated by the parathyroid glands, kidneys, and osteoclasts, and are crucial for proper calcium-mediated metabolism.

Calcium deficiency detected in the blood is often a sign of parathyroid or kidney malfunction since the body always has a large reserve of calcium in the skeletal system. Vitamin D or magnesium deficiency or excessive sodium, phosphorus, or protein consumption may also lead to low blood calcium levels. The RDA for calcium ranges from 200 mg/day in newborn infants to 1200 mg/day in adults. Inadequate calcium intake may lead to osteoporosis, especially in older individuals.

While much of our calcium often comes from dairy products, certain plant products have calcium that is as readily absorbed and utilized as dairy-derived calcium. Kale, broccoli, bok choy, cabbage, collards, and mustard greens are rich plant sources of such calcium. Some plants such as spinach and rhubarb, and to a lesser extent sweet potatoes and kidney beans, are rich in oxalic acid, a potent inhibitor of calcium absorption, thus excessive consumption of any of these foods should be avoided to prevent inhibition of calcium absorption. Phytic acid, found in non-fermented grain products and bean products, also inhibits calcium absorption. Fermented grain and bean products are much lower in phytic acid, since yeast used in the fermenting process metabolizes phytic acid.

Chromium

Chromium is considered an essential mineral, however, why it is essential is still unclear (Higdon *et al*., 2012). Trivalent chromium is the form found in most foods and utilized by the body. Hexavalent chromium at low levels can be reduced to trivalent chromium by acids in some foods and the stomach. However, hexavalent chromium at high levels is a potent carcinogen.

Chromium enhances the effects of insulin on glucose metabolism by enhancing cellular insulin receptors or increasing insulin-stimulated glucose movement into cells. Since chromium has an effect on glucose metabolism, it may also improve blood lipid profiles. There is no RDA for chromium, but rather, an AI which ranges from 0.2 μg/day for newborn infants to 25 μg in adult females and 35 μg/day in adult males. Women who are pregnant require 30 μg/day while women who are breastfeeding require around 45 μg/day. Claims that chromium picolinate supplements enhance weight loss are unfounded (Volpe *et al*., 2001) and may even cause weight gain in some individuals with type 2 diabetes taking sulfonylurea drugs (Martin *et al*., 2006).

Information on the chromium content of foods is limited. Broccoli is particularly high in chromium while green beans, potatoes, grape juice, orange juice, apple, and banana contain moderate amounts. Foods that are high in simple sugars are usually low in chromium and they also are known to promote chromium loss from the body.

Copper

Copper exists as both monvalent and divalent cations in the human body, however, the divalent form is predominant (Higdon *et al*., 2012). Copper easily accepts and donates electrons making it important in oxidation–reduction reactions and free radical scavenging. A number of enzymes called cuproenzymes contain copper and are important in a variety of metabolic activities. One such enzyme, cytochrome c oxidase is important in generating the electrical gradient in mitochondria which makes ATP production possible. Another cuproenzyme, lysyl oxidase, is needed for cross-linking collagen and elastin, important for making strong connective tissue. Copper is also important in iron metabolism, the synthesis and metabolism of neurotransmitters, synthesis and maintenance of the myelin sheath, melatonin synthesis, and regulation of gene expression. Two forms of the powerful antioxidant enzyme superoxide dismutase contain copper.

The RDA for copper is from 200 μg/day in newborn infants to 900 μg/day in adults. Women who are pregnant or breastfeeding require slightly more copper, around 1000 and 1300 μg/day, respectively. Good plant sources for copper include cashews, sunflower seeds, hazelnuts, almonds, peanut butter, lentils, and mushrooms.

Fluoride

Fluoride is a negatively charged molecule of fluorine mostly found in teeth and bones (Higdon *et al*., 2012). It is not considered an essential mineral since it is not required to sustain life, however, it is extremely important in preventing tooth decay. Fluoride interacts with hydroxyapatite in teeth and hardens tooth enamel making teeth less susceptible to decay. No RDA for fluoride exists, however, an AI has been established ranging from 0.01 mg/day for newborn infants to 3 mg/day for adult females and 4 mg/day for adult males. Fluoride is often consumed in fluoridated drinking water, usually between 0.7 and 1.2 mg/l. Excessive consumption of fluoride by infants can lead to dental fluorosis, a whitish speckling of permanent teeth that may lead to staining and pitting in severe cases. Fluoride-containing toothpastes are also a major source of fluoride in children, since they are more likely to swallow the toothpaste rather than spit it out. Tea and grape juice are relatively high in fluoride compared with other plant sources, but even so, both contain very little fluoride (0.6 mg/100 ml serving).

Iodine

Iodine is a non-metallic trace mineral found mostly in ocean water that is required by humans for the synthesis of the thyroid hormones triiodothyrine and thyroxine which are involved in regulating growth, development, metabolism, and reproductive functions (Higdon *et al*., 2012). Iodine deficiency is a worldwide problem, leading to enlarged thyroid (goiter) in children and adults, and when deficient in pregnant women or newborn infants, impaired intellectual development.

The RDA for iodine ranges from 110 μg/day in newborn infants to 150 μg/day in adults. Women who are pregnant or breastfeeding require 220 and 290 μg/day, respectively. Plant-based foods particularly rich in iodine include seaweeds, navy beans, and potatoes. The absolute iodine content of these foods varies widely due to differences in soil and seawater iodine concentrations. In many regions of the world, iodine is supplied through iodized salt. Some foods contain substances called goitrogens that interfere with the uptake of iodine. Cassava, some species of millet, and many cruciferous vegetables contain goitrogens. Several of the soy-based isoflavones including genistein and daidzein inhibit thyroid hormone synthesis. The negative impacts

of goitrogens and soy isoflavones are only of concern in areas where iodine deficiency is severe or these products are consumed in excess.

Iron

Iron is a key mineral influencing metabolism in all living organisms as it is a major component of many proteins and enzymes (Higdon, *et al*., 2012). Iron is a key component of heme compounds, molecules involved in many metabolic functions. Hemoglobin is important in the transport and storage of oxygen in the blood while myoglobin is involved in transport and storage of oxygen in muscles. Cytochromes, molecules containing hemes, are important in mitochondrial electron transport and energy production. Both catalase and peroxidase contain hemes and both are important as antioxidant enzymes, protecting cells from damage by hydrogen peroxide. The immune system also relies on a heme-containing enzyme myeloperoxidase, which is produced by white blood cells which engulf bacteria and kills them by exposing them to ROS. Iron may also be important to physiological adaptations to low oxygen concentrations, such as in high altitude environments, or in patients with chronic lung disease. Iron is also required for DNA synthesis. Since iron can be toxic to cells through generation of free radicals, the human body closely regulates the iron status of our cells, particularly by the enzyme hepcidin which is produced by the liver. Hepcidin inhibits the release of iron from specific cell types that store significant amounts of iron. When iron in the blood is sufficient, hepcidin levels are high, and when iron levels are low, hepcidin levels decrease, allowing iron to be released from storage sites.

Iron deficiency in humans may be one of three types. The first is where storage pools have been depleted, however, there is still enough iron in the blood for normal metabolism. Early functional iron deficiency is the second type. This occurs when the amount of iron in the blood limits the formation of red blood cells, but not low enough to detect anemia. The third level of deficiency, called iron-deficiency anemia, occurs when: (i) blood levels of iron reach a critical level where normal red blood cell formation cannot occur; (ii) red blood cells are smaller than normal; and (iii) their hemoglobin content is lower than normal. At this stage, the oxygen carrying capacity of the blood is compromised and iron-dependent enzyme function is deficient. Symptoms of anemia include those that are concomitant with low blood oxygen levels, such as fatigue, rapid heart rate, and rapid breathing upon exertion.

The RDA for iron varies considerably with age and gender and ranges from 7 to 18 mg/day. Women who are between menarche and menopause generally need 18 mg/day while pregnant women need 27 mg/day. The iron content of food may be of the heme type (hemoglobin and myoglobin, from animal-based foods) and non-heme type (plant sources). The absorption of non-heme iron depends greatly on absorption enhancers and inhibitors consumed with iron-containing food. Non-heme iron absorption is enhanced by vitamin C, organic acids such as citric, malic, tartaric, and lactic acids, and meat, fish, or poultry. Absorption is inhibited by phytic acid (found especially in legumes, grains, and rice), polyphenols, and soy protein. Good plant sources of iron include black-strap molasses, raisins and prunes, potatoes with skin, kidney beans, lentils, tofu, and cashews.

Magnesium

Magnesium is involved with over 300 essential metabolic functions in the human body (Higdon *et al*., 2012). Magnesium is important in the synthesis of ATP, nucleic acids, carbohydrates, lipids, and the antioxidant glutathione. Magnesium also plays a role in transport of ions across cell membranes, and for the phosphorylation of proteins that is important in cell signaling. Magnesium is also part of structural molecules in bone, cell membranes, and chromosomes.

The RDA for magnesium ranges from 30 to 420 mg/day depending on gender and age and deficiencies are rather rare in generally healthy individuals. Green leafy vegetables are a great source of magnesium owing to their high chlorophyll content. Grains and nuts as well as lima beans, okra, molasses, and bananas are also high in magnesium.

Manganese

Manganese is an essential mineral that is part of some enzymes and an activator of others (Higdon *et al*., 2012). Manganese is part of an important antioxidant enzyme manganese superoxide dismutase, found in the mitochondria which are particularly susceptible to free radical damage. Manganese-containing enzymes are involved in

gluconeogenesis, and liver detoxification of ammonia. Manganese-activated enzymes are important in the metabolism of carbohydrates, collagen (important in wound healing), amino acids, cholesterol, and neurotransmitters. Manganese is a cofactor for enzymes involved in cartilage formation.

Manganese deficiency is extremely rare and no RDA has been established. The AI ranges from 0.003 mg/day for newborn infants to 2.3 mg for adults, and varies with age and gender. Plant-based foods rich in manganese include pineapple, whole grains, nuts, leafy vegetables, and teas. Foods that are high in phytic acid, such as beans, seeds, nuts, whole grains, and soy products, or foods high in oxalic acid, such as cabbage, spinach, and sweet potatoes, may slightly inhibit manganese absorption. The tannins in tea may also reduce the absorption of manganese.

Molybdenum

Molybdenum functions as a cofactor for three important enzymes in the human body: (i) sulfite oxidase; (ii) xanthine oxidase; and (iii) aldehyde oxidase (Higdon *et al*., 2012). Sulfite oxidase is important in the metabolism of the sulfur-containing amino acids methionine and cysteine. Xanthine oxidase is important in regulating the antioxidant capacity of the blood and aldehyde oxidase is important in the metabolism of drugs and toxins.

The RDA for molybdenum ranges from 17 to 45 μg/day depending on age and gender. Molybdenum deficiency is extremely rare. The best plant sources of molybdenum are legumes (beans, peas, and lentils), grains, and nuts. Most fruits and vegetables are low in molybdenum.

Phosphorus

Most of the phosphorus in the human body occurs as phosphate and is found in the bone in the form of hydroxyapatite (Higdon *et al*., 2012). Phosphorus is also an important component of membranes, being part of the phospholipid complex producing the membranes' fluid nature. Energy production relies on the phosphorus-rich AMP (adenosine monophosphate), ADP, and ATP family of molecules. Nucleic acids are rich in phosphorus, the buffering system of cells relies on phosphates and many enzymes, hormones, and cell signals rely on phosphorylation for activity.

Phosphorus deficiency is rare but increased consumption of fructose can lead to excessive urinary loss of phosphorus leading to a net daily loss of phosphorus from the body, especially in males (Milne and Nielsen, 2000). This is important since the consumption of high fructose corn syrup has skyrocketed in some areas of the world. The RDA for phosphorus ranges from 460 mg/day in young children, peaking at 1250 mg/day for adolescents lowering to 700 mg/day for adults. Dairy foods, meat, and fish are rich in phosphorus, many food additives contain phosphorus and phosphoric acid is present in soft drinks. The phosphorus in plant-based foods occurs as phytic acid. Only about 50% of the phosphorus in phytic acid is bioavailable to humans since we lack the enzymes required for liberating phosphorus from the phytate. Almonds, peanuts, and lentils are particularly rich in phosphorus.

Potassium

Potassium is important in human nutrition as both an essential mineral and as an electrolyte (Higdon *et al*., 2012). Electrolytes in the body can conduct electricity. Since many bodily functions rely on electrical impulses traveling throughout the body, extremely precise regulation of electrolyte levels is imperative for good health. Electrical charges throughout the body rely on potassium and sodium ions. Potassium ions are principally intracellular while sodium ions are predominantly extracellular. The general gradients of these two ions are such that there are approximately 30 times the potassium ions inside versus outside the cell and ten times the number of sodium ions outside than inside the cell. These differences create an electrical gradient called the membrane potential. The gradients are maintained by ATP-driven membrane pumps which use between 20 and 40% of the energy consumed by an adult at rest. This gives an idea of how crucial these gradients are. The gradients are particularly important for nerve impulse travel, heart function, and muscle contraction. Potassium is also important in enzyme function, particularly for enzymes involved in carbohydrate metabolism.

Potassium deficiency is usually caused by excessive excretion of potassium in the urine rather than a lack of potassium in the diet. There is no RDA for potassium, but rather an AI which ranges from 400 mg/day in infants to 4700 mg/day in adults. Breastfeeding

women require slightly more, around 5100 mg/day. Fruits and vegetables are particularly good sources of dietary potassium, especially bananas, potatoes with the skin, prunes, oranges, tomatoes, raisins, artichoke, lima beans, acorn squash, spinach, sunflower seeds, almonds, and molasses.

Selenium

Selenium is a trace mineral required by humans for specialized enzymes called selenoproteins (Higdon *et al.*, 2012). While a number of selenoproteins have been identified, the function of many of them remains unknown. The main identified function for many of the selenoproteins is that of an antioxidant or an antioxidant generator. Selenoprotein P found in plasma helps protect the lining of blood vessels from the damage caused by peroxynitrite, a reactive nitrogen species. The generation of the biologically active thyroid hormone triiodothronine also requires a selenoprotein. Other selenoproteins have roles in spermatogenesis, protein folding as well as inflammatory and immune responses.

Selenium deficiency doesn't appear to cause a specific illness, but rather renders the deficient individual more susceptible to stress-induced illnesses. The RDA for selenium ranges from 20 μg/day in children to 55 μg/day in adults. Women who are pregnant or breastfeeding require 60 and 70 μg/day, respectively. The best sources for selenium are organ meats and seafood. Plants are unreliable for selenium nutrition as they do not have a specific selenium requirement and merely absorb selenium that is in the soil. Levels of selenium in any particular plant-based food are entirely dependent on the soil in which it was grown. Brazil nuts are a good source of plant-based selenium, but their content can vary from 10 to 100 μg per nut depending on where it is grown.

Sodium chloride

Sodium chloride provides sodium and chloride ions, the principal extracellular ions in the human body (Higdon *et al.*, 2012). Their concentrations are carefully regulated within the body and an excess of either ion can lead to serious health problems. Both sodium and chloride ions are electrolytes which are critical for generating and maintaining membrane potentials in the body for the transmission of nerve impulses, heart function, and muscle contraction. Sodium is important in the absorption of chloride, amino acids, glucose, and water in the small intestine. Chloride is an important component of hydrochloric acid in the stomach, crucial for proper digestion. Sodium is intricately involved in blood volume and blood pressure, with excess sodium leading to high blood pressure and its negative health consequences.

Sodium chloride is normally not deficient in human diets. On the contrary, the major concern with both nutrients, sodium in particular, is an excess. Rather than an RDA, there is an AI and a maximum limit for sodium consumption. The AI is meant as a guide for the minimum amount of sodium required to replace that lost in sweat each day. This amount ranges from 0.3 g sodium chloride/day for infants to around 4 g/day for adults. The maximum recommended salt consumption by adults is 5.8 g/day (sodium chloride) (Food and Nutrition Board, Institute of Medicine, 2005). Processed foods are particularly high in sodium chloride, and excessive salt intake should be avoided by everyone.

Zinc

Zinc plays a very important role in growth and development, immune responses, neurological function, and sexual reproduction (Higdon *et al.*, 2012). Zinc deficiency is an important problem, especially in developing countries. Zinc is an important catalyst for innumerable enzymes. Zinc is also an important structural component in proteins and cell membranes. Proteins containing zinc are important in DNA transcription, cell signalling, and apoptosis.

The RDA for zinc ranges from 3 mg/day for children to 8–11 g/day for adults. Pregnant and breastfeeding women require an additional 3–4 g/day. Shellfish and red meats are good sources of zinc. Nuts and legumes are good plant sources of zinc, however, there is less bioavailable zinc in plant sources due to the presence of phytic acid.

Phytonutrients

Phytonutrients are substances produced by plants that are implicated in maintaining good health or improving poor health (Fig. 17.7). Most phytonutrients are not considered as essential as defined for other nutrients. There are many products with claims of great benefits and the decision of whether or not to use them is daunting to say the least. With the easy

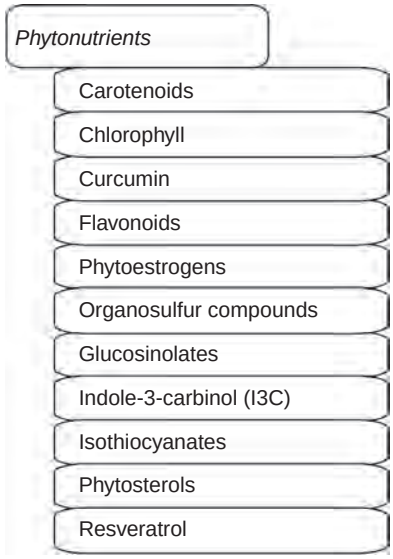


Fig. 17.7. The most commonly studied phytonutrients. Be aware that there are thousands of potential phytonutrients in the foods we eat; they just haven't been discovered yet.

and often immediate availability of information on the Internet, it is relatively easy to research specific claims and determine their validity.

This section explores the major phytonutrients and the latest information available regarding their potential benefits and the risks associated with their use. A wonderful website for information regarding many aspects of nutrition is the Linus Pauling Institute (2013) at Oregon State University (http://lpi.oregonstate.edu/infocenter/). This website and associated references were the sources for much of the material presented in this chapter.

Carotenoids

There are more than 600 naturally occurring pigments produced by bacteria, algae, and plants that are classified as carotenoids (Higdon *et al*., 2012). These pigments are yellow, orange, and red and are often found in abundance in most fruits and vegetables. Green leaves are often rich sources of carotenoids; they aren't brightly colored because the chlorophyll in them masks the other pigments. The carotenoids most consumed by humans include the carotenes (alpha-carotene, beta-carotene, lycopene) and xanthophylls (beta-cryptoxanthin, lutein, zeaxanthin). Carotenoids are fat soluble, thus they must be consumed with fat to be absorbed by the body.

The most widely documented function of carotenes is as components of provitamin A. Of the six listed carotenoids, only alpha-carotene, beta-carotene, and beta-cryptoxanthin are provitamin A carotenoids than can be converted by the body into retinol (vitamin A) (Food and Nutrition Board, Institute of Medicine, 2000). The vitamin A activity of beta-carotene in food is only 1/12 that of retinol. The vitamin A activity of alpha-carotene and beta-cryptoxanthin are both 1/24 that of retinol (Food and Nutrition Board, Institute of Medicine, 2000).

In plants, all carotenoids function as effective antioxidants, especially lycopene. Whether or not they have the same capacity in humans is not clear. Lutein and zeaxanthin are very effective in absorbing blue light. In our eyes, both pigments absorb blue light before it reaches the rods and cones and may protect them from oxidative damage induced by light (Krinsky *et al*., 2003).

Carotenoids stimulate the synthesis of a group of proteins called connexins that form pores in membranes that allow intercellular communication via the movement of small molecules between cells (Bertram, 1999) helping cells stay in a differentiated state. Cancer cells often lose the capacity to stay differentiated. While dietary carotenoids might be able to reduce the risk of cancer, in particular, lung cancer (Voorrips *et al*., 2000; Holick *et al*., 2002), the benefit is small (Gallicchio *et al*., 2008) and the best protection against lung cancer is not smoking. Beta-carotene supplements were actually found to increase the risk of lung cancer in high risk individuals, such as smokers and asbestos workers (Anonymous, 1994; Omenn *et al*., 1996).

While the consumption of tomatoes and cooked tomato products (exceptionally high in lycopene) has been suggested to reduce the risk of prostate cancer in men, the evidence supporting such a claim is limited. In several studies, a significantly decreased risk for prostate cancer was observed in men consuming large amounts of tomatoes and tomato products (Mills *et al*., 1989; Giovannucci *et al*., 1995; Gann *et al*., 1999; Giovannucci, 2002). However, in other studies, high dietary lycopene intake (mostly from tomatoes and tomato products) did not reduce the risk of prostate cancer (Schuurman *et al*., 2002; Etminan *et al*., 2004; Key *et al*., 2007).

Higher blood levels of carotenoids have been associated with lower measures of carotid intima-media thickness, a measure of cardiovascular risk (Iribarren *et al*., 1997; D'Odorico *et al*., 2000; Rissanen *et al*., 2000, 2003; McQuillan *et al*., 2001; Dwyer *et al*., 2004). Studies evaluating decreased risk of cardiovascular disease and plasma carotene content vary, with some suggesting a decreased risk (Street *et al*., 1994; Rissanen *et al*., 2001; Sesso *et al*., 2004; Ito *et al*., 2006; Buijsse *et al*., 2008) while others suggesting no effect (Sahyoun *et al*., 1996; Evans *et al*., 1998; Hak *et al*., 2003; Sesso *et al*., 2005). Consumption of foods rich in carotenoids seems to lead to a decrease risk of cardiovascular disease (Rimm *et al*., 1993; Gaziano *et al*., 1995; Sahyoun *et al*., 1996; Osganian *et al*., 2003). Since consumption of carotenoid-rich foods leads to reduced risk but higher plasma levels of carotenes are not associated with the decreased risk, other factors associated with the consumption of carotenoid-rich food (such as lifestyle, other nutrients) must be involved. Beta-carotene supplements do not offer the same protection as food-derived carotenoids (Voutilainen *et al*., 2006).

Even though many fruits and vegetables are good sources of carotenoids, many of the carotenoids they contain have limited bioavailability due to protein association within the food. Chopping and cooking often release bioavailable carotenoids and bioavailable lycopene from tomatoes is substantially increased if the tomatoes are cooked with a little oil. Pumpkin and carrots are particularly rich in alpha-carotene while pumpkin, spinach, sweet potato, carrots, collards, kale, and turnip greens are rich sources of beta-carotene. Remember alpha- and beta-carotene are both provitamin A carotenoids that can be converted into retinol. Beta-cryptoxanthin, another provitamin A carotenoid, can be found in many orange and red fruits including pumpkin, red peppers, and papayas.

Good sources of lycopene include tomatoes, tomato products, and watermelon. Lutein and zeaxanthin are both xanthophylls and their levels are typically reported combined. Foods rich in lutein and zeaxanthin include spinach, kale, turnip greens, and collards.

Chlorophyll and chlorophyllin

Chlorophyll is the major light-capturing pigment in plants. It closely resembles the hemoglobin in our bodies in that it has in its structure a central porphyrin ring (Higdon *et al*., 2012). In chlorophyll the center of the ring is magnesium while the center in hemoglobin is iron. The two main types of chlorophyll, a and b, are both fat-soluble molecules, situated predominantly in the chloroplast membranes of leaf cells. The difference between chorophyll a and chlorophyll b is that they each absorb light of different wavelengths. Chlorophyllin is a synthetic mixture of chlorophyll and copper salts that is often taken as a supplement.

Both chlrophyll and chlorophyllin form molecular complexes with suspected carcinogens, most notably certain hydrocarbons found in tobacco smoke, compounds found in cooked meat, and aflatoxin-b1, a potent liver carcinogen found in moldy grains and legumes. Combined with chlorophyll or chlorophyllin, these potential carcinogens may be less easily absorbed during digestion, reducing the chances for cancer. Chlorophyllin has been shown to: (i) inhibit the activity of some enzymes involved in the development of cancer (Tachino *et al*., 1994; Yun *et al*., 1995); (ii) neutralize the oxidative capacity of suspected chemical carcinogens and radiation (Park *et al*., 2003; Kumar *et al*., 2004); and (iii) arrest the development of colon cancer cells (Chimploy *et al*., 2009).

Chlorophyllin is often taken as a supplement to act as an internal deodorant for individuals with colostomies, ileostomies, or incontinence to reduce urinary and fecal odor. Clinical trial results are inconclusive as to whether odor is reduced with chlorophyllin supplementation (Higdon *et al*., 2012). A mixture of papain (an enzyme derived from papaya), urea and chlorophyllin seems to enhance wound healing and reduce the odor associated with severe, slow-healing wounds (Smith, 2008).

Leafy greens such as spinach, kale, collards, and herbs such as parsley and basil are the best natural sources of chlorophyll.

Curcumin

The rhizomes of *Curcuma longa*, a relative of ginger, are the raw material from which the spice turmeric is derived (Higdon *et al*., 2012). Turmeric's bright yellow color comes from curcuminoids, fat-soluble polyphenols. Turmeric extracts are often used as food-coloring agents. Curcumin is the most abundant curcuminoid in turmeric.

Detection of cucurmin in the human body after oral ingestion is generally limited to the gastrointestinal tissues where it accumulates. Metabolites

of curcumin such as curcumin glucuronides, curcumin sulfates, and hexahydrocurcumin are readily detected in the bloodstream (Lao *et al*, 2006; Baum *et al*., 2008) but are much less effective than curcumin itself. Only low levels of cucurmin can be detected in the bloodstream after ingestion (Cheng *et al*., 2001; Sharma *et al*., 2004). Curcumin is a powerful antioxidant, however, limited translocation may reduce its effectiveness outside the gastrointestinal tract (Garcea *et al*., 2004). Curcumin also enhances the production of the antioxidant glutathione (Dickinson *et al*., 2003; Zheng, *et al*., 2007). Curcumin reduces symptoms of inflammation (Deodhar *et al*., 1980; Satoskar *et al*., 1986) by interfering with enzymes responsible for producing the irritants associated with inflammation (Hong *et al*., 2004). Curcumin has also been shown to arrest cell development in cultured colon cancer cells (Moos *et al*., 2004; Tsvetkov *et al*., 2005) and cultured breast cancer cells (Somasundaram *et al*., 2002). While these responses to curcumin appear promising, it is important to emphasize the very low bioavailability of curcumin outside the gastrointestinal tract. Additionally, the cancer studies were performed using cell cultures, not patients. Curcumin is ingested as the spice turmeric or taken as a supplement.

Flavonoids

Flavonoids are water-soluble polyphenolic pigments synthesized by plants (Higdon *et al*., 2012). Their many functions in plants include: (i) floral pigmentation to attract pollinators; (ii) stimulating *Rhizobium* bacteria for nitrogen fixation; (iii) promotion of pollen tube growth; (iv) regulation of auxin accumulation; (v) regulation of the resorption of mineral nutrients from senescing leaves; (vi) enhancing tolerance to abiotic stress; (vii) absorbing otherwise damaging UV radiation; (viii) acting as antioxidants; (ix) providing defense against herbivores and pathogens; and (x) promoting allelopathic relationships with other plants.

Flavonoids are often divided into subclasses to include anthocyanidins, flavanols, flavanones, flavonols, flavones, and isoflavones. While flavonoids are ubiquitous in plants, some plants are particularly rich in one or more of the flavonoid classes. Anthocyanidins (cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin) are found in red, blue and purple berries, red and purple grapes, and red wine. Flavanol monomers or catechins (catechin, epicatechin, epigallocatechin epicatechin gallate, epigallocatechin gallate) are found in green and white teas, chocolate, grapes, berries, and apples. The flavonol classes of theaflavins and thearubigins are found in black and oolong teas while the proanthocyanidin flavonols are found in chocolate, apples, berries, red grapes, and red wine. Citrus fruits and juices are particularly rich in flavanones (hesperetin, naringenin, and eriodictyol). Flavonols (quercitin, kaempferol, myricetin, and isorhamnetin) can be found in yellow onions, scallions, kale, broccoli, apples, berries, and teas. Flavones (apigenin and luteolin) are found in parsley, thyme, celery, and hot peppers. Soybeans and soy products as well as other legumes are high in isoflavones (daidzein, genistein, and glycitein).

When flavonoid molecules are attached to one or more sugar molecules they are called flavonoid glycosides. Those that are not attached to sugar molecules are called aglycones. The flavonoids we consume are all of the flavonoid glycoside type except for catechins and proanthocyanidins (Williamson, 2004). Most flavonoid glycosides must be metabolized by intestinal bacteria before being absorbed. Regardless of their form, flavonoids are rapidly eliminated from the body, thus studies linking them to specific health benefits must be considered carefully. In addition, the biological activity of metabolites is often not the same as those of the parent compound from which they were derived.

Contrary to popular belief, flavonoids are probably not very important to human health when it comes to their antioxidant activity (Frei and Higdon, 2003; Williams *et al*., 2004; Lotito and Frei, 2006). Levels of other antioxidants such as vitamin C, uric acid, and glutathione are often 100–1000 times higher than flavonoids in the bloodstream. Additionally, the little flavonoids found in the blood are usually metabolites rather than the parent flavonoid. Similarly, it is not known whether or not flavonoids are effective as metal chelators in the body (Frei and Higdon, 2003).

Flavonoids are very important in cell signaling pathways (Williams *et al*., 2004) and may help prevent cancer by: (i) enhancing the excretion of potential carcinogens (Kong *et al*., 2001; Walle and Walle, 2002); (ii) preserving normal cell cycle regulation, limiting the production of mutations (Chen *et al*., 2004; Wang *et al*., 2004); (iii) inducing apoptosis in cancer cells (Kavanagh *et al*., 2001; Sah *et al*., 2004; Ramos, 2007); and (iv) inhibiting tumor invasion and the development of tumor

blood vessel networks, known as angiogenesis (Kim, 2003; Bagli *et al*., 2004).

Flavonoids may reduce the susceptibility to cardiovascular disease by: (i) decreasing inflammation (Cho *et al*., 2003; Sakata *et al*., 2003; O'Leary *et al*., 2004); (ii) reducing the expression of adhesion molecules by vascular wall cells, one of the first steps in the development of atherosclerosis (Choi *et al*., 2004; Ludwig *et al*., 2004); (iii) maintaining vasodilation (Anter *et al*., 2004); and (iv) decreasing platelet aggregation and the formation of blood clots (Bucki *et al*., 2003; Deana *et al*., 2003).

Soy isoflavones (phytoestrogens)

Soy isoflavones receive a discussion separate from other flavonoids due to their importance as an estrogen mimicking hormone. Since they are plant-derived compounds that exert estrogen-like activity, they are called phytoestrogens (Lampe, 2003). Soybeans are the primary legumes containing phytoestrogens. The phytoestrogens in soybeans are isoflavones attached to sugar molecules, isoflavone glycosides, which include genistin, daidzin, and glycitin. Once fermented or digested, the sugar molecule is removed leaving isoflavone aglycones, genistein, daidzein, and glycitein, respectively.

How the soy isoflavones are metabolized depends on the bacteria in the human intestine (Rowland *et al*., 2003). For example some individuals metabolize daidzein to equol, a compound with greater estrogenic activity than daidzein, while others metabolize it to other less estrogenic compounds, all depending on intestinal flora (Setchell *et al*., 2002). An individual gut health and bacterial profile can have great implications for their response to isoflavone ingestion.

Soy isoflavones mimic estrogen, a signaling molecule (hormone) responsible for many aspects of human function, especially heart, liver, bone, brain, and reproductive growth and development. Estrogen works by attaching to estrogen receptors in cells to form an estrogen–receptor complex which then interacts with DNA and alters the expression of estrogen-sensitive genes. Soy isoflavones can bind to estrogen receptors and mimic estrogen effects in some tissues and block estrogen-like effects in others. Soy isoflavones are of interest for their potential for: (i) reducing the risk of certain hormone-related cancers such as breast, uterine and prostate cancers; (ii) enhancing bone density; and (iii) improving blood lipid profiles, particularly cholesterol levels. Soy isoflavones and their metabolites also inhibit the synthesis and activity of some enzymes involved in estrogen metabolism and may alter the biological activity of both estrogens and androgens (Kao *et al*., 1998; Whitehead *et al*., 2002; Holzbeierlein *et al*., 2005). Isoflavones may also inhibit cell proliferation and act as an antioxidant.

Consuming isoflavones may improve cardiovascular health by lowering serum LDL cholesterol (Sacks *et al*., 2006) and decreasing arterial stiffness (Nestel *et al*., 1997). While breast, uterine and prostate cancer rates often appear to be lower in populations consuming significant amounts of soy isoflavones (Goodman *et al*., 1997; de Kleijn *et al*., 2001; Horn-Ross *et al*., 2003; Messina, 2003; van Erp-Baart *et al*., 2003; Xu *et al*., 2004), there is little direct evidence that consuming soy isoflavones reduces one's risk for these diseases (Murray *et al*., 2003; Goetzl *et al*., 2007). Similarly, populations consuming soy foods generally have a lower incidence of hip fracture, suggesting greater bone density in those individuals. However, it is not clear whether or not consumption of soy isoflavones improves one's bone density profile (Setchell and Lydeking-Olsen, 2003). Use of isoflavones rather than estrogen therapy to counter symptoms of menopause, particularly hot flashes, has not been particularly effective (Krebs *et al*., 2004). However, women who produce equol from ingested isoflavones experienced a significant reduction in the occurrence of hot flashes (Jou *et al*., 2008).

It is important to note that not all soy products contain isoflavones, therefore it is important when considering the possible benefits associated with soy consumption, that the products include isoflavones. Some soy products rich in isoflavones include soy protein concentrate prepared via an aqueous wash (as opposed to an ethanol wash), miso, tempeh, boiled soybeans, dry roasted soybeans, soymilk, and tofu. Many soy-based infant formulas are high in isoflavones (Setchell *et al*., 1998). The isoflavone content of soy-based foods can vary considerably even within different lots of a single brand (Setchell *et al*., 2001). Supplements containing isoflavones are not standardized and quality control is an issue with many available on the market (Setchell *et al*., 2001; Chua *et al*., 2004), thus care should be exerted when considering such products.

Garlic (organosulfur compounds)

Garlic (*A. sativum* L.) is an especially rich source of organosulfur compounds. These compounds are responsible for the strong flavor of garlic as well as its possible health benefits (Block, 1985). There are two main classes of organosulfur compounds in garlic: (i) gamma-glutamylcysteines; and (ii) cysteine sulfoxides. The gamma-glutamylcycsteine content is not altered by crushing, chopping or chewing raw garlic. Allylcysteine sulfoxide (alliin) is the predominant cysteine sulfoxide in garlic. When raw garlic is crushed, chopped or chewed the enzyme alliinase is released, converting alliin to allicin in 10–60 s (Block, 1985). Allicin then breaks down over time into a number of organosulfur compounds.

Allicin and allicin-derived compounds are rapidly metabolized by the human body (Lawson, 1998) perhaps into allyl methyl sulfide, which is readily detected in the breath. Gamma-glutamylcysteines are absorbed then hydrolyzed to *S*-allylcysteine and *S*-1-propenylcysteine (Jandke and Spiteller, 1987; de Rooij *et al*., 1996).

Garlic may be good for cardiovascular health. The consumption of garlic and derived organosulfur compounds appears to decrease the synthesis of cholesterol by liver cells (Gebhardt and Beck, 1996) by inhibiting enzymes responsible for its production (Liu and Yeh, 2002; Ferri *et al*., 2003). Organosulfur compounds from garlic also inhibit platelet aggregation in lab tests (Chan *et al*., 2002). Cardiovascular disease is at least in part caused by inflammation and garlic has been shown to inhibit two enzymes in the inflammatory response pathway (Ali *et al*., 2000), and to decrease the production of inflammatory signaling molecules *in vitro* (Keiss *et al*., 2003; Chang *et al*., 2005). Hydrogen sulfide may act as a vasodilator, thereby protecting heart health (Pryor *et al*., 2006; Lefer, 2007).

Organosulfur compounds may help the body prevent activation of the carcinogenic capacity of some toxins as well as rid itself of potentially carcinogenic toxins (Loizou and Cocker, 2001; Gurley *et al*., 2002; Chen *et al*., 2004; Fisher *et al*., 2007). They may also act as antioxidants and stimulate the production of the antioxidant glutathione (Banerjee *et al*., 2003). Organosulfur compounds also induce cell cycle arrest in cancer cell cultures (Knowles and Milner, 2001; Herman-Antosiewicz and Singh, 2004; Arunkumar *et al*., 2006) thereby preventing further unregulated cell division. These compounds also induce apoptosis in pre-cancerous and cancerous cells (Balasenthil *et al*., 2002) which are normally resistant to apoptosis (Wu *et al*., 2005). Sulfur compounds are also antibacterial and antimicrobial (Fenwick and Hanley, 1985; Harris *et al*., 2001).

The most potent source of these organosulfur compounds is chopped, crushed or chewed raw garlic. Cooking garlic inactivates the alliinase enzyme, thus if garlic must be cooked for consumption, allow it to stand for 10 min after chopping to allow the alliinase enzyme to convert alliin to allicin (Song and Milner, 2001).

Glucosinolates

Cruciferous vegetables are rich sources of sulfur-containing compounds called glucosinolates. Diets rich in cruciferous vegetables seen to reduce the risk of several types of cancer (Verhoeven *et al*., 1997).

INDOLE-3-CARBINOL Many cruciferous vegetables are rich sources of glucobrassicin (Kim and Milner, 2005). When these vegetables are chewed or chopped, indole-3-carbinol (I3C) is enzymatically produced from glucobrassicin by myrosinase, an enzyme that is normally isolated from glucobrassicin in the plant cell. When I3C hits the acidic environment of the stomach, a number of acid condensation products are formed including the dimer 3,3-diindolylmethane (DIM) and a cyclic trimer (CT) which are the substances responsible for biological reactions attributed to the consumption of cruciferous products. These acid condensation products are less likely to form if the vegetables are cooked since myrosinase is inactivated by heat and any I3C formed by intestinal bacteria is not likely to form condensates in the alkaline environment of the intestine.

The active components of I3C condensation seem to interfere with the transformation many potential carcinogens (pro-carcinogens) must undergo in the body before they become carcinogenic (Wallig *et al*., 1998; Bonnesen *et al*., 2001; Nho and Jeffery, 2001). In addition, I3C and DIM have been shown to induce apoptosis in cultured prostate (Chinni *et al*., 2001), breast (Hong *et al*., 2002; Howells *et al*., 2002; Rahman and Sarkar, 2005), pancreatic (Abdelrahim *et al*., 2006), and cervical cancer cells (Chen *et al*., 2004). They may also inhibit angiogenesis (Chang *et al*., 2005; Wu *et al*., 2005), required for tumor growth.

Rich sources of glucobrassicin include broccoli, Brussels sprouts, cabbage, cauliflower, collard

greens, kale, mustard greens, radish, rutabaga, and turnips. Glucobrassicin is water soluble and myrosinase is inactivated by cooking. Both of these factors should be considered in the preparation of any cruciferous vegetable for consumption.

ISOTHIOCYANATES Isothiocyanates are hydrolysis breakdown products of glucosinolates, catalyzed by the enzyme myrosinase. Each glucosinolate has a different breakdown product and specific foods are often rich in one particular glucosinolate. For example broccoli is rich in glucoraphanin and sinigrin, precursors to the isothiocyanates sulforaphane and allyl isothiocyanate, respectively. Watercress is rich in gluconasturtiin which is converted to phenethyl isothiocyanate while garden cress is rich in glucotropaeolin, which yields benzyl isothiocyante. All of the isothiocyanates seem to have possible anti-carcinogenic properties.

Isothiocyanates appear to interfere with the transformation of pro-carcinogens into carcinogens (Hecht *et al*., 1995; Hecht, 2000; Conaway *et al*., 2002). Many isothiocyanates protect DNA from damage caused by carcinogens and ROS (Kensler and Talaway, 2004; Zhang, 2004; Fimognari and Hrelia, 2007). They also induce cell cycle arrest in cultured cells (Zhang, 2004) and induce apoptosis in cultured cancer cells (Hecht, 2004). Cell cycle arrest is an important stage of the normal cell cycle required for normal cell development. Isothiocyanates may also decrease the secretion of inflammatory signaling molecules (Heiss *et al*., 2001; Gerhauser *et al*., 2003). They are also fairly effective as an antibacterial agent towards *Helicobacter pylori*, a bacterial strain associated with an increased risk of gastric cancer (Fahey *et al*., 2002; Normark *et al*., 2003).

Nearly all of the cruciferous vegetables including bok choy, broccoli, broccoli sprouts, Brussels sprouts, cabbage, cauliflower, horseradish, kale, kohlrabi, mustard, radish, rutabaga, turnip, and watercress, are rich sources of the glucosinolate precursors of isothiocyanates (Fenwick *et al*., 1983). The amount of active isothiocyanates derived from each food depends on preparation and cooking methods.

Lignans (phytoestrogens)

Lignans are polyphenolic compounds found in many plants. Lignan precursors are found in many of the plant-based foods we eat and are converted by bacteria in the intestines into enterodiol and enterolactone (Lampe, 2003) where they are then absorbed into the bloodstream. The quantity of enterodiol and enterolactone derived from lignan precursors depends on the microflora in the gut. Both enterodiol and enterolactone mimic estrogen in the human body, thus their precursors are called phytoestrogens. Even though phytoestrogens from soy seem to have received the most attention as phytoestrogens, especially in the popular press, lignan precursor phytoestrogens are equally important, especially in Western diets. The lignan precursors identified in the average human diet include pinoresinol, lariciresinol, secoisolariciresinol, and matairesinol.

Enterodiol and enterolactone both have weak estrogenic activity in the human body including effects on bone, liver, heart, brain, and reproductive health similar to those of soy isoflavones. Lignans may alter endogenous estrogen activity (Brooks and Thompson, 2005) and can act as antioxidants. Diets that are rich in lignans are associated with a reduced risk of cardiovascular disease. Many of the foods containing significant lignans are also rich in other nutrients and phytonutrients which may also contribute to their cardioprotective status. There is little evidence of reduced risk of breast, uterine and prostate cancer with increased consumption of lignan-rich foods. This is not to convey that lignan-rich foods may not reduce the risk of these cancers, just that there is not sufficient evidence yet to make such a statement.

The best source of lignans is ground flax seed. Flaxseed oil is not a rich source of lignans; crushed whole seeds are the source for flax lignans. Other good sources include: (i) pumpkin, sunflower, poppy, and sesame seeds; (ii) rye, oats, and barley; (iii) bran from wheat, oat, and rye; (iv) beans; (v) berries; and (vi) vegetables.

Phytosterols

Phytosterols are plant-derived substances that mimic the structure and function of cholesterol. Plants produce many different sterols. Two main classes of phytosterols are recognized: (i) sterols which have a double bond in the sterol ring; and (ii) stanols which lack the double bond. The most abundant sterols in the human diet are sitosterol and campesterol. Stanols are also present in plants but at much lower levels.

Phytosterols inhibit the absorption of cholesterol in the intestines and reduce both total and LDL

serum cholesterol, reducing the risk of cardiovascular disease (Katan *et al*., 2003; Berger *et al*., 2004). In addition, sitosterol has been shown to induce apoptosis in cultures of human prostate (von Holtz *et al*., 1998), breast (Awad *et al*., 2003), and colon (Choi *et al*., 2003) cancer cells.

All plant-based foods contain phytosterols with the highest levels in unrefined plant oils including corn, soy, peanut, canola, nut, rice bran, and olive oils (Ostlund, 2002). Wheat germ, nuts, seeds, whole grains, and legumes are also very good sources of phytosterols (de Jong *et al*., 2003). Many plant-based margarine spreads are enriched with plant sterols and stanols, providing a convenient way to supplement normal phytosterol intake.

Resveratrol

Resveratrol (3,4′,5-trihydroxystilbene) is a polyphenolic, fat-soluble molecule that occurs in two configurations, *cis* and *trans*, or as glucosides, often produced by some plants in response to stress (Aggarwal *et al*., 2004). *Trans*-resveratrol is readily absorbed by humans when ingested, but it is rapidly metabolized and eliminated from the body (Walle *et al*., 2004; Wenzel and Somoza, 2005). It is important to keep in mind that many of the studies touting the benefits of resveratrol have been performed with resveratrol at 10–100 times the level observed in human plasma immediately after consumption. Human tissues are exposed primarily to metabolites of resveratrol and not resveratrol itself. Very little is known about the metabolic activity of resveratrol metabolites.

While there are some claims that resveratrol is a powerful antioxidant, there is not much evidence that resveratrol is an important *in vivo* antioxidant (Bradamante *et al*., 2004). Resveratrol may or may not influence estrogen metabolism (Tangkeangsirisin and Serrero, 2005) and may have anti-inflammatory properties (Pinto *et al*., 1999; Donnelly *et al*., 2004).

Resveratrol may help prevent cancer since it has been shown to: (i) increase the transformation of potentially carcinogenic chemicals to excretable forms in cultured cells (Jang *et al*., 1997; Yang *et al*., 2003); (ii) induce cell cycle arrest in cancer cell culture (Joe *et al*., 2002); and (iii) inhibit proliferation of cancer cells in culture and induce apoptosis in them (Aggarwal *et al*., 2004). Resveratrol has also been observed to inhibit angiogenesis *in vitro* (Igura *et al*., 2001; Lin *et al*., 2003; Chen and Tseng, 2007). Again, whether or not these observations can be made *in vivo* remains to be seen, especially considering the fact that resveratrol is quickly metabolized and many human tissues are never likely to be exposed to resveratrol levels used in many studies.

Resveratrol may reduce cardiovascular risk by: (i) reducing vascular cell adhesion (Ferrero *et al*., 1998; Carluccio *et al*., 2003), one of the earliest events in atherosclerosis; (ii) inhibiting the proliferation of vascular smooth muscle cells (Haider *et al*., 2003; Mnjoyan and Fujise, 2003), another component of atherosclerosis; (iii) stimulating arterial relaxation (Wallerath *et al*., 2002; Klinge *et al*., 2005); and (iv) inhibiting platelet aggregation (Pace-Asciak *et al*., 1995; Kirk *et al*., 2000). Resveratrol is the component in red wine that many have suggested explain the "French paradox" of low coronary heart disease despite the consumption of high levels of saturated fat and extensive cigarette smoking. While some component of red wine or lifestyle associated with red-wine drinkers may account for at least some of the paradox, there is still much work that needs to be done to establish even limited causality.

Some studies with yeast, worms (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*) and fish (*Nothobranchius furzeri*) have indicated that resveratrol seems to extend the lifespan by a mechanism similar to caloric restriction (Wood *et al*., 2004; Valenzano *et al*., 2006).

Resveratrol is found in grapes (skins only), peanuts, blueberries, and cranberries (Sanders *et al*., 2000; Rimando *et al*., 2004).

Summary

The information available these days regarding what we should and should not eat to achieve and maintain our best health is staggering. All plant-based products that have been implicated in human health concerns could simply not be covered in a text such as this. Rather, a general summary of the most up-to-date information on the major nutrients, minerals, and phytonutrients was presented to stimulate interest in this exciting area of research. The takeaway message from this chapter is be careful what you eat, eat the freshest food you can possibly find (or better still, grow your own), and prepare it in such a way as to minimize the loss of nutrients from it. Eat the whole food, not just a part of it, it'll do you good.

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