Ren Sen Zeng • Azim U. Mallik • Shi Ming Luo

Editors

Allelopathy in Sustainable Agriculture and Forestry

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Preface

The idea of putting together this volume stemmed from a successful International Symposium on Allelopathy Research and Application held in Sanshui, Guangdong, China during April 27–29, 2004. The symposium was supported by several agencies: National Natural Science Foundation of China (30410303040), Department of Science and Technology of Guangdong Province, Sanshui Municipal Government and South China Agricultural University. Eighty five researchers from eight countries working on various aspects of allelopathy attended the symposium. The National Natural Science Foundation of China (30424006) encouraged and supported the book publication. Selected papers presented at this symposium and some additional invited chapters constitute this volume. Several edited books and reviews on allelopathy have appeared at the turn of the last century. Why another book on allelopathy? It is largely because the discipline has been experiencing extraordinary growth in research and there is a great deal of interest in seeking alternative environmentally friendly methods of weed control in agriculture, ways to deal with replant problems and soil sickness in horticulture, and competition control in young plantations. Research and application of allelopathy can help find some of the desirable alternatives. Our understanding in allelopathy mechanisms has increased significantly with use of recently developed sophisticated tools and techniques in biochemistry, molecular biology and genetic engineering. Researchers in allelopathy are taking advantage of these rapidly expanding fields of molecular biology in understanding the complex plant–plant and plant-microbe interactions in seeking solutions to agricultural problems. We felt that it is timely to collect and synthesize the latest developments on allelopathy research with special emphasis on its application in sustainable agriculture and forestry. We divided the contents of the book into three sections: (i) past and recent history of allelopathy, (ii) allelochemicals and allelopathic mechanisms, and (iii) application of allelopathy in agriculture and forestry. We thank the authors who responded to our call and contributed to these topics. Most importantly we are grateful to the reviewers for spending their valuable time in making critical comments on these chapters. Shekhar Biswas was helpful in editing some figures and putting together all the chapters in the appropriate format. We thank Springer for accepting our proposal for this book, especially Jinnie Kim for keeping faith in us despite the long delay in completing this volume.

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Participants of the International Symposium on Allelopathy Research and Application held in Sanshui, Guangdong, China during April 27–29, 2004

Contents

Part 1 History

Part 2 Allelochemicals and Allelopathic Mechanisms

Part 3 Application of Allelopathy in Agriculture and Forestry

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Introduction: Allelopathy Research and Application in Sustainable Agriculture and Forestry

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The evolutionary history of agriculture is intimately associated with anthropogenic manipulation of plants and ecosystems in order to increase yield and quality of crops. Superior seed selection, hybridization and innovative cultural practices such as disease and weed control, irrigation and soil fertility management by crop rotation, mulching and manuring all have contributed significantly to increased agricultural production. The dramatic increase in yield of selected food crops has been achieved in the second half of the 20th century following the so called green revolution created by the development and extensive use of high yielding variety (HYV) seeds, irrigation water and a wide variety of agrochemicals as fertilizers, paste and weed control agents in highly mechanized farming system giving birth to a new form agriculture, the Industrial Agriculture. However, the yield increase in this mode of agriculture came with a terrible cost of environmental degradation and this high-input, high-yield industrial agriculture has proven to be unsustainable (Allison and Hobbs 2007).

The single most important attraction in this form of highly mechanized and high input agriculture is increased productivity. Sustainable or not "more resources now" draws instant attention in our world of over six billion people even if it is not sustainable in the long run. Ecological degradation resulting from this form of agriculture is extensive, expensive and often beyond repair. Industrialized agriculture is performed in a business model where maximizing crop production at all costs is the preoccupation just like maximizing profit is the preoccupation in business. In this form of agriculture for the sake of increasing efficiency large tracts of land are brought under a single farm by homogenizing diverse habitats that often cause loss of soil organic matter, soil structure and above and belowground biotic diversity (Drinkwater and Snapp 2007). Land degradation by salination and water logging are common in intensively irrigated cropland across the world. In addition, farmer's poor FTT put them out of operation resulting in social decline (Allison and Hobbs 2007). This "one size fits all" approach of industrial agriculture characterized by extensive use of HYV seeds, capital intensive farm machineries, a large variety of agrochemicals and irrigation water does not take into account the unique ecological conditions of the diverse habitats, sociological, cultural and economic standing of the farmers. Inherent in this mode of agriculture is the false assumption that the use of genetically modified seeds and agrochemicals will continue to maintain high productivity and satisfy the increasing food demand. It assumes that the high external agricultural inputs will replace the need for internal feedbacks of the soil system. Bluntly put, the idea of continuously growing high yielding crops under completely weed-free conditions (the modern day herbicide ready crops) is madness and makes no ecological sense. This mode of agriculture does not take into account the ecological costs of growing crops and it is neither ecologically or economically sustainable.

Agricultural crops and systems must be developed based on ecological status of the natural capital of the area such as excess, optimum or limitation of water, soil nutrients, human capital such as labor, trained agricultural personnel and economic conditions of the farmers. Indigenous crops evolved under local ecological conditions can be improved and cultivated by modern scientific methods for local consumption and sale as opposed to growing HYV crops that are developed under superfluous nutrients, water and disease protection. These plants are not geared to cope with the local natural environmental conditions and require heavy external inputs that are often scarce in developing countries. This has caused disruptions in cropping patters that had been in place for centuries and created ecological degradation of precious lands.

An alternative form of agriculture must be developed that is not only productive but also ecologically and economically sustainable and socially just. In this system farmer and policy makers' aspirations must be matched with the ecology of the land and appropriate technology that is ecologically sustainable and economically viable for achieving long-term sustainability. Ecological solutions to modern agricultural problems particularly environmental degradation must be found. It begs the question, are there better ways to enhance the productivity of crops within the ecological bounds of its habitats? The central premise of the concept of ecological agriculture is just that. Crop production within the bounds of ecological thresholds will not only ensure sustainability but also preserve the diversity of culture and heritage among this society. We need to develop ways and means to practice agriculture in an ecological framework and not in a business framework as is currently practiced. In this context can the science and application of allelopathy help? Simply put, allelopathy refers to an ecological phenomenon of plant–plant interference through release of organic chemicals (allelochemicals) in the environment. These chemicals can be directly and continuously released by the donor plants in their immediate environment as volatiles in the air, root exudates in soil, or the microbial degradation products of plant residues. The chemicals may interfere with survival and growth of neighboring or succeeding plants. Black walnut, eucalyptus, sunflower, sesame and alfalfa are common examples of plants with allelopathic property including staple crops such as rice, wheat, barley and sorghum. Plants can emit chemicals that also discourage insects and pathogens. To maintain sustained productivity, knowledge of his form of plant interference on other plants and on disease causing organisms has been used in agriculture since prehistoric time by manipulating the cropping pattern and sequence such as mixed cropping and crop rotation. More than 2000 years ago, ancient Chinese, Greeks and Romans observed the phenomenon of plant chemical interference (although they did not call it alleopathy) and applied the knowledge for sustaining crop yield, controlling weeds, diseases and pests. But nowadays the use of numerous agrochemicals including a wide range of herbicides, pesticides, fertilizer and genetically modified crops has become the characteristic feature of modern agriculture. Not only the sustainability of crop yield is called into question in this form of agriculture, but the extensive long-term and often irreversible environmental degradation including ground water contamination and food safety associated with industrial agriculture are now serious concerns world wide. Although crop yield may not be as high as in industrial agriculture, for the interest of sustained yield, food safety and environmental protection many traditional societies still use crop rotation, mixed cropping and intercropping, which is also gaining popularity in the industrial world by the name of organic agriculture.

Apart from the deterioration of agricultural environments due to agrochemicals, forest management practices using herbicide and pesticide in young plantations is a cause for concern. In intensively managed forests fertilizer application is also common. Adverse effects of herbicides on wild berries, other non-timber plant and animal resources and water quality have been documented (Moola et al. 1998; Arimond 1979; Lautenschlager 1993; Balfour 1989; Bouchard et al. 1985; Neary and Micheal 1996). As 80% of fresh water comes from forest, preserving water quality alone is a good reason for avoiding chemical control of weeds and insects in forestry practices. Knowing the adverse effects of herbicides and pesticides on the environment and wildlife, herbicide and pesticide use in forest management has been banned in some jurisdictions such as the province of Quebec in Canada and sub-Alpine regions of southern France. Public support is at an all time low in chemical weed and insect control in forestry (Wagner et al. 1998). Research on alternative methods of weed and pest control in forestry is underway. Several studies indicate that there is a strong potential for developing allelopathic methods of weed control in forestry (Jobidon 1989, 1991; see also Mallik, for a review in this volume). Thermal decomposition of allelochemicals by forest fires and adsorption of undecomposed allelochemicals in charcoal has been elucidated by Wardle et al. (1998). This emphasizes the role of wild fires in removing allelopathy from forest floor humus in fire-adapted ecosystems. Forests harvesting followed by prescribed burning can be used as a means to reduce competition as well as forest floor allelochemicals. Another approach in dealing with forest allelopathy is mycorrhizal inoculation of tree seedlings. Laboratory and greenhouse studies have shown that certain conifer ectomycorrhizae can not only degrade and detoxify allelochemicals but they can also use these chemicals as their carbon source (Zeng and Mallik 2006). There is a good opportunity to exploit this aspect of allelopathy research to develop practical methods for enhancing crop tree seedling growth where the use of fire is not possible.

Although the phenomenon of allelopathy has been known and used in agriculture since the ancient time its use in land management has been rather limited. Recent advances in ecological understanding and methodological breakthroughs in biochemical analysis and molecular techniques provide unprecedented opportunity to discover new methods and tools to exploit and use allelopathy principles in sustainable agriculture. In many natural ecosystems allelopathy plays a major role in structuring plant community and succession by influencing soil chemistry and nutrient dynamics, controlling competitive interactions and productivity of desirable plants (Vivanco et al. 2004). A wide variety of allelochemicals are involved in interactions between plants and microbes, such as fungistasis and spore germination, antibiosis between microorganisms, development of disease symptoms, promotion of infection and host resistance to pathogens. So far applied research in allelopathy has been mostly targeted for weed control. The potential for allelopathic plants and microorganisms to control crop diseases, increase disease resistance in crops through signal transduction, development of biocontrol agents, biofumigants and plant growth promoters is endless but little explored.

In a recent review Birkett et al. (2001) explored if allelopathy offers a real promise for practical weed and pest management. They particularly focused on rhizospheric studies of plant signaling compounds involving allelopathy and concluded that in addition to the bulk allelochemical effects of certain plants and microbes in the environment, the signaling property of certain allelochemicals can be exploited for practical weed management. Through a deeper understanding and manipulation of rhizosphere ecology especially by making use of the dynamics of signaling molecules to our advantage we can open a new era of sustainable crop production. With respect to specific examples of practical weed and pest control by allelopathy, Birkett et al. (2001) cite several examples: (i) Exploitation of traditional intercropping approaches with plants such as *Mentha* spp., *Saturega montana* and cultivated members of the genus *Ocimum* to suppress weeds (Shlevin 2000). The essential oils from these plants are applied in soil to replace the commonly used methylebromide. Successful control of noxious weeds and parasitic plants such as stigas (*Sriga asiatica* and *S. hermonthica*) has been reported by intercropping even with aggressive competitors such as sweet potato (*Ipomaea batatus*) (Oswald et al. 1998). (ii) Two leguminous intercropping plants, silverleaf (*Desmodium uncinatum*) and greenleaf (*D. intortum*) are not only effective in controlling *S. hermonthica* infestation in corn (*Zea mays*) but they also work against stem borer of corn because of their insect repelling property (Khan et al. 2000). Farmer-managed field trials have shown highly significant increases in corn yield (almost double) by intercropping corn with these legumes compared to the traditional intercropping with hemp (*Crotolaria* spp.), soybean (*Glycine* spp.) and cow-pea (*Vigna unguiculata*). Khan et al. (2000) suggested that the mechanism involved is principally allelopathy in addition to nitrogen fixation. (iii) Use of leguminous cover crops with allelopathic property such as velvetbean (*Mucuna pruriens*) has been proven beneficial in rice cultivation and corn-kidney intercropping systems in Japan (Fujii 2003; Fujii et al. 1991a,b; Azumi et al. 2000). The author attributed the increased yield in rice to nitrogen fixation and allelopathy of the legume. (iv) Germination stimulants and other regulatory signal compounds produced by allelopathic plants can influence crop-weed interaction.

Our 10 000 years history of agriculture is replete with examples of ancient traditional biological and cultural methods of crop protection. Refinement of many traditional techniques of crop rotation, mixed cropping, mulching, cover cropping, green manuring and microbial inoculation can enhance crop productivity without compromising food safety and environmental quality. Many plants and microbes can be used to control agricultural weeds and pests. Over the last four decades a large number of allelopathy bioassays and small-scale experimental trials have been conducted. Time has come to use these results to demonstrate the applicability of this knowledge by conducting statistically designed large-scale field trials involving farmers. At the same time it is essential to have a greater understanding of molecular biology and rhizosphere ecology of crops and weeds with allelopathic property by elucidating the molecular mechanisms of plant–plant and plant-pathogen interactions that are directly associated with sustainable agriculture and forestry.

The objective of this volume is to report on the latest advances in alleopathy by inviting leading scientists to contribute in specific fields. The content of this volume is divided into three themes: (i) historical aspects of allelopathy, (ii) allelochemicals and allelopathic mechanisms, (iii) application of allelopathy in agriculture and forestry. Following a general introduction each theme was developed with contributions from several authors.

Aliotta et al. (Chapter 1) discussed allelopathy in the context of ancient agricultural writings of Greeks and Romans. These authors also discussed the allelopathic effects of several Mediterranean plants and the use of olive water mill waste as a potential bio-herbicide and insecticide. Mallik (Chapter 2) reviewed the recent history of allelopathy, challenges and opportunities of this discipline while Zeng (Chapter 3) focused on the history of allelopathic studies in Chinese agriculture. An et al. (Chapter 9) described concentration dependent allelopathic response by mathematical modeling and they compared that with ancient Chinese Yin/Yang philosophy of duality in unity. Haig (Chapter 4) reviewed the chemistry of diverse plant allelochemicals. Weir and Vivanco (Chapter 5) reviewed the methods for determining the interacting nature of allelopathic effects using biochemical and molecular techniques, Weidenhamer reported on the development of the dynamic assessment of allelopathy in soil systems (Chapter 6) and Molecular mechanisms of allelopathy were explored by Duke et al. in Chapter 8. Castells (Chapter 7) reviewed indirect effects of phenolic compounds on plant performance through changing microbial activity and thereby decreasing soil N availability. Several authors reviewed the significance of allelopathy in important crops such as rice (Kim and Shin, Chapter 10; Shen et al. Chapter 11), wheat (Wu et al. Chapter 12), sorghum (Cheema et al. Chapter 13) and soybean (Yan and Yang Chapter 14). Liu et al. reviewed the autotoxicity phenomenon in agriculture and forestry in Chapter 15 while black walnut alleopathy in agroforestry was reviewed by Jose and Holzmueller (Chapter 16). B. Mallik and Williams reviewed the stimulatory effects of allelopathy involving microorganisms in Chapter 17. Use of allelopathic cover crops for weed control and roadside restoration was reviewed by Weston and Eom (Chapter 18) and the book ends with a review of the role of disturbance and allelopathy in forestry (Chapter 19).

In this volume the phenomenon of allelopathy has been addressed from historical, fundamental mechanistic and applied perspectives with selected examples. We ought to remind ourselves of its prevalence in natural and managed ecosystems, its complexity and potential application in land management. A better understanding of this phenomenon would enable us to make meaningful advances in sustainable resource management.

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Part 1 History

1. Historical Examples of Allelopathy and Ethnobotany from the Mediterranean Region

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Abstract. The true scientific study of plants began as a consequence and a part of the great intellectual movement of the sixth century BC in Asia Minor and in the Mediterranean Region. Greek and Roman scholars viz., Theophrastus, Cato the Elder, Varro, Vergil, Columella and Pliny the Elder wrote treatises on agriculture dealing with aspects of good crop husbandry used to minimize weed interference with crops by hand weeding, mechanical methods, tillage, burning and mulching. They were the forerunners of allelopathy. In this chapter we combined our expertise in reviewing the agricultural and ethnobotanical knowledge of ancient Greeks and Romans. In particular we focused our attention on medicinal and edible plants (rue, olive, squill and lavender). Their ethnobotanical information suggests great opportunities for the future through full evaluation of their potential allelochemicals in plant interactions.

1.1 Introduction

About three-quarters of the biologically active plant-derived compounds presently in use worldwide have been discovered through follow-up research to verify the authenticity of data from folk and ethnomedicinal uses (Farnsworth 1990). Plants have always been significant in every culture; their uses include food, medicine, fiber, fuel, building materials and many other purposes. Nowadays, cultural and demographic changes threaten the conservation of this patrimony of knowledge. In the last decades, the growing appreciation of the value of traditional knowledge prompted the revival of ethnobotanical research.

Since medicinal plants discovered by traditional societies are proving to be an important source of potentially therapeutic drugs, the interest of many researchers has been focused mainly on this subject. Very little ethnobotanically-directed research has been conducted in order to optimize the exploration for novel allelochemicals of potential use in agriculture. These fields have in common an important trait: the biological activity of a plant extract either for medicinal purposes or for the control of undesired plant growth and that is mainly due to the occurrence of a particular set of secondary products in a given plant. Thus, in principle, it is conceivable that a plant that is highly appreciated in ethnomedicine could also be useful in agriculture, for its weed control property.

In general, traditional knowledge is orally transmitted from generation to generation, and ethnobotanical research plays an important role in documenting and describing folk uses of plants. In the Mediterranean Region, the analyses of the writings of ancient Greece and Rome, together with standard ethnobotanical research, could result in a fundamental source of information on the large body of empirical knowledge of plant products and their potential uses in agriculture to control the undesired growth of weeds. This approach of study through ancient text was first proposed by Holland (1994), who suggested re-examining the extremely large and readily accessible corpus of classical medical treatises in search of new drugs based on phytochemicals. Although fascinating, the proposal introduced some problems that still deserve consideration (Piomelli and Pollio 1994). The first is that there are only a limited number of old technical texts, and even when adequate critical editions exist, few translations are available, making these treatises available only to scholars with a solid background in Greek or Latin. The second problem is one of plant identification. It is well known that ancient texts provide no iconography and limited description of plants; for this reason the identity of the plants mentioned in the texts is always a controversial matter. For example, according to Buenzen (2004), the identification of the 257 plant-based drug prescriptions in the *Corpus Hippocraticum* is uncertain for only 11 plants, whereas Raven (2000) is clearly skeptical about the possibility of resolving the identity of a Greek phytonim down to the species level, in accordance with Linnaean nomenclature. The last problem deals with the interpretation of the techniques described, which are often an assembly of empirics, philosophy and folklore and are not easy to interpret.

The same cautious approach should be undertaken in the study of ancient writings in search of allelochemicals from Mediterranean plants, keeping in mind that this research needs a multidisciplinary effort involving specialists from diverse disciplines, such as phylogeny, botany, ecology and history of agriculture. In this chapter we review the agricultural and ethnobotanical knowledge of ancient Greeks and Romans. We particularly focus on medicinal and edible plants of the Mediterranean region as their ethnobotanical descriptions may offer great opportunities to fully evaluate their potential allelochemicals in plant interactions.

1.2 Agricultural Knowledge in Ancient Greece and Rome

While some brief remarks of the time can be appropriated here, an elaborate treatment of this aspect is beyond the scope of this article. Cato, Varro and Columella wrote for the guidance of big land-owners cultivating with large gangs of slaves, but Vergil wrote for the gentleman-small-holder. Cato's work consists of brief notes of guidance for the practical farmers, without art of ornament, clearly revealing his hard arid character, little softened by his insistence on the unfailing value of cabbage as both food and medicine. Both Varro and Columella produced systematic treatises that are, for us, the primary sources of information on agriculture of their time. The Georgics of Vergil remains unique. A supreme artistic creation, inspired by the ancient scientific and agricultural tradition as expressed by Epicurus and Lucretius, it contrives also to convey a wealth of practical farming instruction. The direct influence of Theophrastus is evident in many places (Morton 1981).

1.2.1 Enquiry into Plants and Causes of Plants (Theophrastus 1980)

Theophrastus (ca. 370–285 BC) was born at Eresos on the island of Lesbos and is believed to have gone to Athens as a young man to study at the Academy. In 323 BC he became the director of Lyceum when his mentor Aristotle retired. The first sentence of Theophrastus' Enquiry into Plants (*Historia Plantarum*) proclaims that the study of plants has become a science. "*We must consider the distinctive characters and the general nature of plants from the point of view of their morphology, their behavior under external conditions, their mode of generation, and the whole course of their life*". The relation between wild and cultivated plants, which was of intense interest to the ancient botanists (partly for religious reasons), is discussed by Theophrastus, who considered that man's special care (pruning, grafting, weeding etc.), not divine intervention, produced cultivated plants from the wild. Theophrastus was the first to report allelopathy, which Molisch investigated 2000 years later: "*some things though they do not cause death enfeeble the tree as to the production of flavours and scents; thus cabbage and sweet bay have this effect on vine. For they say that the vine scents the cabbage and is affected by it wherefore the vine shoot, whenever it comes near this plant, turns back and looks away as though the smell was hostile to it*" (*Enquiry into Plants,* Book IV, XVI, 5).

1.2.2 Liber de Agricultura (Cato 1979)

Cato the Elder (234–140 BC), the famous Roman politician and writer was a farmer during his youth. He wrote the first Latin treatise on agriculture. In this book Cato reported that chickpea (*Cicer arietinum* L.), barley (*Hordeum vulgare* L.), fenugreek (*Trigonella foenum-graecum* L.) and bitter vetch (*Vicia ervilia* L.) all "scorch up" corn land (*De Agricultura*, XXXVII).

1.2.3 *De Re Rustica* (Varro 1979)

Marcus Terentius Varro (116–27 BC) was considered as the most erudite man and a most prolific writer of his times. De Re Rustica libri III (three books of farming) is one of the most important books of its kind extant from antiquity. Very interesting is Varro's definition of agriculture as "not only an art but also a science which instructs us what crops should be sown in each type of soil and what should be done so that the land may continue to give the highest yields" (I, 3).

1.2.4 *Georgics* (Vergil 1989)

Vergil (70–19 BC) was one of the most famous Roman poets. In his Georgics Vergil reported: Urit essim lini campum seges, urit avenae, urunt Lethaeo perfusa papavera somno; sed tamen alternis facilis labor. Flax (*Linum usitatissimum* L.), oat (*Avena sativa* L.) and papaver (*Papaver rhoeas* L.), parch the soil; however crop rotation reduces the problem (Georgics 1:77). Vergil's reference to the important practice of seed selection is noteworthy: "I have seen farmers treating seeds with salnitre and pomace so that they were going to be bigger than ineffective ones, becoming soft even with cool temperature. Seeds select with great accuracy have been lost. Therefore human wisdom has to select the bigger ones every year. If it does not happen, every thing inevitably goes wrong." (Georgics 1, 193–200). This is not referred to by Theophrastus, although it must have been established from very ancient times and would be well known to him.

1.2.5 De Re Rustica (Columella 1994)

Written by Lucius Columella, native of Cadiz (Spain), in the first century AD and retaining its authority throughout the Middle Ages, this treatise is the longest and most systematic account of Roman agriculture and is an example of the Roman genius at its best (Morton 1981). In the introduction Columella pointed out the decline of agriculture*: "... and agriculture, which the best of our ancestors treated with the best attention, we have handed over to the very worst of our slaves as if to the hangman for punishment"* (Liber I, 3). It is interesting what Columella wrote about the techniques to preserve quinces and apples. *"...these are well preserved in boxes made of lime tree or beech wood; the boxes are kept in cool and dry environments: The apples are disposed on a sheet of paper with the stalk side up. One must make sure that fruits do not make contact with one another. Different varieties of apples must be kept in different boxes. In fact, when different varieties are kept in the same box, they do not get along with one another, and they get spoiled soon"* Liber (XII, 47). Molisch (1937) coined the term allelopathy in describing this phenomenon.

1.2.6 *Naturalis Historia* (Pliny 1938–1963)

The *Naturalis Historia* of Pliny the Elder (23–79 AD) is the most important encyclopedia about the scientific knowledge of plants and it consists of 37 books, of which 16 are on botany. He reported that chickpea produces salty seeds that scorch up the soil (Book XVIII: 124), bitter vetch (*Vicia ervilia* L.) grown in a vineyard, causes the withering of the vines (Book XVIII: 138) and the "shade" of walnut (*Juglans regia* L.) is "*heavy, and even causes headache in man and injury to anything planted in the vicinity and that of the pine tree also kills grass*" (Book XVII: 89). He also stated that "*All elements for the shadow of walnut tree or stone pine or a spruce or silver fir to touch any plant whatever in undoubtedly poison*". He used the term "shade" in a broad sense, to include the usual concept of partial exclusion of light, effects on nutrition and chemicals that escape from the plants into the environment.

The influence of Romans treatises on agriculture was enormous for many centuries. Collections of these agricultural writing were frequently published as far back as Renaissance and is often referred to as *Scriptores rei rusticae.* They still represent an invaluable source of knowledge for the present plant scientists searching the development of early agriculture.

1.3 Brief Overview of the Mediterranean Vegetation and Some Historical Examples of Its Allelopathic Plants

The Mediterranean vegetation is quite different from that of any other European Region. It is characterized by evergreen trees, shrubs and bushes that can survive long and very hot summers without rain. Most weedy plants, during summer, dry and remain inactive with dormant buds in the soil, while annual plants end their life cycle in the summer. Moreover, the ancient settlements on the Mediterranean coasts have deeply modified the natural vegetation. Today's woods survive in locations that are not accessible to men and animals, along with dense evergreen communities, called maquis or, more frequently with dwarf evergreen shrubs, the so-called phrygana. Most of the woody species have hard and thick leaves, which reduce their transpiration in dry summers. Another characteristic is the abundance of aromatic plants that release essential oils and are deterrents for the pasture (Polunin and Walters 1985).

In our allelopathic studies to isolate potential bioherbicides from plants, we focused on ancient medicinal plants and vegetable wastes, which represent a primary and yet neglected source of allelochemicals. Here we summarize some studies on important plants of Mediterranean Region such as rue, olive, squill and lavender, which can be used as potential natural pesticides in sustainable agriculture.

1.3.1 Ethnobotany of Rue

Ruta graveolens L. (Rutaceae), or common rue, originating in Southern Europe, is an evergreen shrub with bluish-green leaves that emits a powerful odour and has a bitter taste. The plant is cited in the ancient herbals and has deep roots in folklore, alchemy and even demonology. Rue has been regarded from the earliest time as successful in warding off contagion and preventing the attacks of fleas and other noxious insects. The name rue derives from the Greek "reuo" (= to set free), because the plant is efficacious in various diseases. Rue was the chief ingredient of the famous antidote to poison used by Mithridates. It was also known to produce erythema and pustular eruptions on human skin. Many remedies containing rue as well as its abortive properties were mentioned by Pliny the Elder in his *Naturalis Historia* (XX, 143). In Europe, rue was considered a powerful defense against witches during the Middle Ages. Piperno, a Neapolitan physician, in 1625, recommended rue as a treatment for epilepsy and vertigo. Today, the aerial parts of the plant are eaten in Italian salads, and are said to preserve the eyesight. Rue is currently mentioned in the pharmacopoeias of 28 countries where it is considered mainly as a stimulating, antispasmodic, diuretic and emmenagogue. Moreover, fresh and dried leaves are used to preserve and to flavour beverages and foods such as liquor (grappa) and wine, cheese and meat (Aliotta, Cafiero, Oliva and Pinto 1996).

1.3.1.1 Potential Use of Rue Allelochemicals as Pesticides

The presence of large amounts of coumarins on the leaf surface of rue and their easy extraction through leaching (Zobel and Brown 1988) has a potential use in agricultural pest management.

Weed Control

Rue contains furanocoumarins, acridone and quinolone alkaloids, and flavonoids and its water extract delays the onset and decreases the rate of crop seed germination both in vitro and under field conditions. The bioassay-oriented study of this extract led us to isolate some active pure compounds: the coumarins 5-MOP, 8-MOP and 4-hydroxy-coumarin and the flavonoid quercetin. These substances also inhibit germination and radicle elongation of radish, zucchini, cauliflower and tomato. Coumarine (5-MOP) from rue inhibits radish seed germination by 86% and radical growth by 87% at 2×10^4 M concentration (Aliotta, Cafiero, De Feo, and Sacchi 1994). Soil incorporated with rue leaves produce higher water-soluble phenolics, electrical conductivity and nitrate, and lower pH values compared to untreated soil (Oliva, Lahoz, Contillo and Aliotta 2002).

Based on results obtained from crop seeds rue extracts were used as a possible bioherbicide against germination of weeds in agricultural soil. Greenhouse experiments were conducted in pots containing field soil to which aqueous rue leaf extracts (10% w/v) were applied. Weed emergence was delayed in the treated pots compared to the control when 10% concentrations of rue extracts were applied to the soil. Purslane (*Portulaca oleracea* L.*)* and purple nutsedge (*Cyperus rotundus* L.) population constituted 95% whereas weed population was less than 5% consisting of a few redroot pigweed (*Amaranthus retroflexus* L.), lambquarter goosefoot *(Chenopodium* album L.), bermuda grass *(Cynodon dactylon* L.) and dwarf spurge *(Euphorbia exigua* L.). Rue water extract increased soil respiration and soil microbial biomass (Aliotta, Cafiero, De Feo, Palumbo and Strumia 1996).

Insect Control

Rue has been traditionally used against insects. Although some of its allelochemicals are involved in plant-insect interactions, the plant did not receive much attention in terms of its potential for insect pest control. We have tested the effectiveness of aqueous rue extract against growth and development of the Mediterranean fruit fly (medfly, *Ceratitis capitata* Wiedmann) and the mosquito larvae (*Culex pipiens* L.). The medflies have migrated around the world and cause serious damage to many crops, fruits and vegetables. Rue extract (10%) added to the artificial diet of medfly showed 100% mortality of medfly eggs, delayed metamorphosis of first instar larvae for two days and produced 26% less pupae than control. Furthermore, most of the pupae from treated first instar larvae failed to produce adults. Rue extract showed marked inhibitory effects on mosquito larvae. Rue leachates at 1 and 2% (w/v) induced 50 and 100% mortality of larvae respectively. Preliminary studies showed that active chemical constituents of rue extract causing the biological activities were flavonoids and coumarins (Aliotta, Cafiero, De Feo, Di Blasio, Iacovino and Oliva 2000).

Fungal Control

In addition to weed control, rue extract and its allelochemicals 8-MOP; 5-MOP and 4-hydroxycoumarin showed significant antifungal property. With in vitro assays we determined that 5 g rue lyophilized extract/L, the lowest concentration tested, inhibited mycelial growth of *Pyrenochaeta lycapersici* and *Verticillium dahlie*, pathogenic fungi, by 63.6 and 47%, respectively, with ICso values of 4.16 and 6.5 g/L, respectively. The 8-MOP and 5-MOP, showed more inhibitory effect than the other coumarines. Hyphal growth inhibitory values of *Fusarium solani* and *Trichoderma viride* (at IC_{50}) were 6 mg/L (5-MOP) and 27 mg/L (8-MOP) for the two fungi respectively (Oliva, Lahoz, Contillo and Aliotta 1999). Antifungal activities of some quinolone alkaloids isolated from an ethyl acetate extract of rue showed moderate activity against *Colletotrichum* sp. and highly active against *Botrytis cinerea* (Oliva, Meepagala, Wedge, Harries, Hale, Aliotta and Duke 2003).

Algal Control

Aqueous extracts of rue were tested on six microalgae strains (UTEX 625 *Synechococcus leopoliensis*; UTEX 1444 *Anabaena flos-aquae*, SAG 11.60a *Chlamydomonas oblonga*; CCAP 202/7a *Ankistrodesmus braunii*; CCAP 211/8h *Chlorella emersonii*; UTEX 1648 *Selenastrum capricornutum*) [30]. Rue extract (1% w/v) drastically inhibited the growth of all the tested strains, except A. *braunii.* However, the growth inhibition was reversible. Samples of each algal culture, collected daily during the course of the experiments, resumed their normal growth rate when they were resuspended in a medium without the rue extract. The rue extract was purified and from the active fractions three flavonoids (7-hydroxy-flavone, rutin, and quercetin) and five coumarin derivatives (5-MOP, 8-MOP, 4-hydroxycoumarin, esculetin, and scopoletin) were isolated. These compounds were assayed in broth medium against the selected algal strains, at concentrations ranging from 10^{-4} to 10^{-6} M. Rutin, quercetin, 7-hydroxyflavone, scopoletin and esculetin had no effect on algal growth whereas 4-hydroxycoumarin showed a weak inhibitory activity on two strains. The most active substances isolated were 5-MOP and to a lesser extent 8-MOP. A decrease in growth rate during the exponential phase of growth was observed when 5-MOP and 8-MOP, at concentrations ranging from 10^{-4} to 10^{-5} M, were applied to algal culture, but the former compound at 10^{-5} M inhibited growth more severely than the latter (Aliotta, De Feo, Pinto and Pollio 1999).

1.4 Ethnobotany of Olive Trees and Olive Oil

Olive *(Olea europaea* L., Oleaceae) is a small, slow-growing tree, which often lives to a great age in fairly well drained soils. The plant originated in the Mediterranean region where its fruit has represented an important crop since ancient times (there are many references to olive trees in the Bible and in classical writings) not only as food but also as a source of edible oil used chiefly for cooking, salads and for canning fish. In some religions olive oil is prescribed for burning in sanctuary lamps and for anointing (Grieve 1967). The annual production of 100 Million liters of olive oil mill waste water is a major environmental problem in the Mediterranean basin. Olive oil is obtained by pressing the ripe fruits. The extraction also generates an aqueous phase formed by the water content of the fruit combined with the water used to wash and process the olives; the combination is the so-called "olive mill waste water" (OMW). The polluting organic load of this wastewater is considerable. It has very high biological oxygen demand (BOD) and chemical oxygen demand (COD) reaching concentrations of 100 kg/m^3 and 200 kg/m^3 respectively, while the organic matter contains sugar, tannins, polyphenols, polyalcohols, pectin and lipids. The phytotoxic and antibacterial effects of olive wastewater have been attributed to their phenolic content.

1.4.1 Allelochemicals from Olive Oil Waste Water

Many evidences show that phenolics are involved in allelopathy. Phenolics are very common in decomposition products of plants and are important precursors of humic substances in soils. In soil phenolics can occur in three forms: free, reversibly bound, and bound forms. The ortho-substituded phenolics such as salicylic and o-coumaric acids, and dihydro-substituted phenolic such as protocatechuic and caffeic acids, are adsorbed by clay minerals by forming chelate complexes with metals. Free phenolic compounds may accumulate in rhizosphere soils especially in soils flooded with vegetable waste waters, influencing accumulation and availability of soil nutrients and rates of nutrient cycling, both of which ultimately affect plant growth. Capasso (1997) reviewed the chemistry, biotechnology and ecotoxicology of the polyphenols naturally occurring in vegetable wastes. In phytotoxicity assay carried out on tomato (*Lycopersicon esculentum*) cv "Marmande" and vegetable marrow (*Cucurbita pepo*) cv "S. Pasquale" plants, cathecol, tyrosol and hidroxytyrosol caused wilting of cuffed leaves after 48 hr. Catechol showed the most deterrent effect on the oviposition of the olive fly *Bactrocera oleae*. One of the best known examples of the protective role of allelochemicals is that of protocatechuic acid, and cathecol in onion, against infection of *Colletotrichum circinaus* (Capasso, Cristinzio, Evidente and Scognamiglio 1992). These water soluble phenolics diffuse out from the dead cell layers of the scales and inhibit spore germination and/or hyphal penetration of the pathogen (Rice 1984). One way to reduce OMW pollution in soil is to filter the waters by the molecular weight of the components and assay their phytotoxicity. Using this technique we recently discovered that among the filtered fractions of OMW, the reverse osmosis fraction is responsible for a strong inhibitory activity on germination of radish, wheat, redroot pigweed and lambsquarter. Olive oil mill wastewaters and their filtered fractions were tested for their phytotoxicity on seed germination and seedling growth of radish and wheat. The most potent inhibition was observed with the reverse osmosis fraction. From the latter 17 polyphenols with molecular weight less than 300 Dalton were isolated and identified. The inhibitory activity of each polyphenol was much lower than that observed with the initial fraction. Seed germination bioassays with a mixture of pure polyphenols present in reverse osmosis fraction, showed that the phytotoxicity is caused due to a synergic action of polyphenols with more polar organic compounds and/or inorganic components (Aliotta, Fiorentino, Oliva and Temussi 2002). Microscopic observations of radish seed and the microalga *Ankistrodesmus braunii* under OMW stress showed different morphological and cytological responses. Light and electron microscopy of radish radicle revealed that cell expansion was reduced and the apex was wider and coarser than that of control; the mitochondria were the only cellular organelles showing structural alterations relative to the control at ultrastructural level. For algal cells, the treatment caused cellular anomalous shapes, reduction of cellular volume, altered cytoplasmic organization and inhibition of endospore production (Aliotta, Ligrone, Ciniglia, Pollio, Stanzione and Pinto 2004).

The polluting reverse osmosis fraction of olive oil wastewater was also examined for its herbicide activity against seed germination of two major weeds: redroot pigweed (*Amaranthus retroflexus* L.) and lambsquarter (*Chenopodium album* L.) after the release of the seed dormancy. The reverse osmosis fraction strongly inhibited seed germination of both weeds, while a synthetic fraction prepared from thirteen pure polyphenols isolated from the original active fraction, resulted less inhibition on weed germination. This phytotoxicity could be due to a synergic action of polyphenols with other unidentified substances present in the wastewater. Microscopic observations showed the seed structure of the two weeds and their germination responses in the presence and absence of reverse osmosis fraction Both seeds of redroot pigweed and lambsquarter are lenticulars (1 mm), their seed coat is frequently covered by residues of the thin papery pericarp, the embryo is curved around the starchy hard perisperm that represents the seed storage reserves. The endosperm is reduced to a thin layer around the embryo. The peripheral face of the seed coat presents an opening: the micropyle. The control seeds began their germination after 24 hr of moistening, and the first structure that protrudes from the micropyle of the seed is the endosperm layer, which cover the radicle. When the germination proceeds further, the radicle breaks the endosperm and protrudes. After 96 hrs seedlings of both species are well developed. By contrast in treated seeds the onset of germination was delayed and no seedlings were developed up to 96 hrs. (Aliotta, Cafiero and Fiorentino 2002).

1.5 Ethnobotany of Squill (*Urginea maritima* (L.) Baker)

Squill is a medicinal and ornamental plant native to the Mediterranean. Squill was valued as a medicine in early classical times. It is considered to be the Sea Onion referred to by Homer in the Odyssey (Book X). Oxymel of Squill, used for coughs, was supposedly discovered by Pythagoras (VI Century BC). Theophrastus referred to squill in both his *History of Plants* and *Causes of Plants* as if it were a common plant. Dioscorides described in detail the uses of Squill bulbs and seeds in the second book of his treatise, dedicated to herbs "with a sharp quality": *Scilla is sharp and burning but it is roasted and made useful for man's purposes... It is given in drinks or in aromatic preparations when we wish to promote the flow of urine, to those with dropsy.* In the Naturalis Historia by Pliny Squill is cited in books XIX, 5, 30, 93; and XX, 9, 39, 97: *Scilla...is drunk in doses of three obolos, with honey and vinegar, to cure hydropisia and stimulate urination.* A preparation of squill with honey was also administered by the Arabic physicians of the Middle Ages, who reintroduced the drug into European medicine. These preparations are still in use (Aliotta, De Santo, Pollio, Sepe and Touwaide 2004).

1.5.1 Potential Use of Squill as Rodenticide and Insecticide

The bulbs of squill were an ancient source of rodenticide products re-placed later on by warfarin and modern anticoagulant raticides. Since rats have developed resistance to such products there is now a renewed interest in the species. In the 1950s attempts were made in the United States to introduce this new crop for arid lands. Scilliroside, a high toxicity bufadienolide glycoside is the main active principle. Other glycosides and aglycones have also been isolated from the bulb. Scilliroside content of bulbs is highest in late summer after a dormancy period and does not appear to change with age. The scilliroside content of seed-derived varieties differs substantially, indicating a genetic factor affecting toxicant levels in the individual seedling plants (Verbiscar, Patel, Banigan and Schatz 1986). Ethanolic extracts of squill bulbs have shown activity against stored product pests such as *Tribolium castaneum* (Coleoptera: Tenebrionidae) such anti-insect effects were attributed to bufadienolides of squill (Pascual-Villalobos 2002).

1.6 Ethnobotany of Lavender (*Lavandula* spp.)

Lavender's medicinal properties have long been recognized. Dioscorides described a Lavender species of uncertain attribution in De Materia Medica. It is likely aspic (*Lavandula spica* L.) and stoechas (*L. stoechas* L) lavender. A lavender infusion of spikes and foliage was administered internally to relieve indigestion, headaches and sore throats. Externally, lavender was used to clean wounds and burns or to treat skin ailments. Pliny the Elder reported that lavender was useful in the treatment of menstrual problems, stomach and kidney ailments, jaundice, dropsy and against insect bites (Grieve 1967). Today, lavender is primarily grown for the oil made from the flowering stalks, used in perfumes, soaps and scented sachets. The oil is also used to give fruity flavors to beverages, baked goods and gelatins, usually at levels below 45ppm. Lavender contains 0.5–1.5% volatile oil, tannins, coumarins, flavonoids and ursolic acid (Duke 1986).

1.6.1 Potential Allelochemicals from Lavender

The allelopathic potential of *Lavandula* spp. (Labiatae) has been studied in phryganic ecosystems by Vokou (1992, 1999). The main goals of her research were: (i) to understand the function of essential oils and explain the predominance of aromatic plants in the Mediterranean environment, and (ii) to explore their possible applications given the biological activity of their allelochemicals. This author showed that the essential oil of lavender was a very potent inhibitor of potato sprouting (Vokou 1992, 1999). Moreover, Karamanoli, Vokou, Menkissoglu and Costantinidou (2000) demonstrated the influence of secondary metabolites on the bacterial colonization of the phyllosphere of four aromatic species of the Mediterranean Region: lavender, sage, oregano and rosemary. Lavender (*Lavandula angustifolia*) was heavily colonized by bacteria, in comparison with the others. The differences in bacterial colonization were related to the plants' content of secondary metabolites and their antimicrobial activity. This lavender has the lowest amount of surface phenolics, the lowest concentration of essential oil and the overall weakest antibacterial activity. The essential oil of Lavender (*Lavandula hybrida* Rev.) has shown insecticidal activity against *Acanthoscelides obtectus* Say. with a LC_{50} ranging from 0.5 to 2.4 mg/L (Papachristos, Karamanoli, Stamopoulos and Menkissoglu-Spiroudi 2004).

1.7 Conclusions

Natural products have been the source of many pesticides, used either directly as crude preparations or as pure compounds. Rather than being used directly, they have been more often used as structural leads for the discovery and development of natural product-based pesticides. There are more examples of natural product use as fungicides, insecticides, and other pesticides than as herbicides (Pachlatko 1998). However, there are some success stories with herbicides, and natural products remain part of the herbicide discovery strategy for those companies that still have herbicide discovery programs. The rationale for natural products in herbicide discovery strategy has several disparate components. Nature is full of bioactive materials and compounds with unexploited properties. Many of the hundreds of thousands of secondary products generated by plants, microbes and animals are the result of co-evolution of the producing organism with pests. Thus, the compounds have biological activity. Sometimes, the function of the compound in nature is as a phytotoxin comparable to that produced by plant pathogens or allelochemicals produced by allelopathic plants. Biocides with a specific function in nature can be used for quite different purposes, as with many natural product-based pharmaceuticals. Furthermore, biological activity is more certain with secondary compounds from nature than with randomly synthesized compounds. Ethnobotany is a promising avenue in the search of natural pesticides. To ascertain the potential of the allelochemicals as an economical tool for pest management in crops (e.g., their biodegradability and selectivity) more research is needed. In this regard bioassay of allelopathic effects of ancient medicinal plants and vegetable waste can be used to determine their efficacy in weed and insect control.

Improved instrumentation has considerably reduced the cost of isolation and identification of natural compounds from what it was a decade ago. This has promoted renewed interest in herbicide discovery from natural products. Another major reason for interest in natural phytotoxins is that they often have novel sites of action (Duke, Dayan, Rimando, Schrader, Aliotta, Oliva and Romagni 2002). Even if a phytotoxin is unsuitable for commercial use, identification of a new molecular target site can be very valuable in the design of synthetic herbicides. Furthermore, natural compounds and their preparations as herbicides and pesticides require less regulatory scrutiny for registration than synthetic compounds, thus reducing the cost of their commercialization.

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2. Allelopathy: Advances, Challenges and Opportunities

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Abstract. The phenomenon of plants influencing neighbouring plants through the release of chemicals in the environment has been known as early as c. 370 BC. Greeks and Romans have used this knowledge in agriculture since c. 64 AD. However, it was not until 1937 when Hans Molisch gave it a formal name, allelopathy. The definition of allelopathy ranges from simple to all-inclusive and complex, creating controversy as to its limits and bounds. The complexity and interacting nature of the allelopathy phenomenon makes it difficult to demonstrate its role in community organization. The challenge is to separate the allelopathic effects from other processes such as competition under field conditions, raising an even greater controversy of its legitimacy. To skeptics it remains a controversial subject that often suffers from inconclusive proof. Nonetheless, it is impossible to deny the existence of plant–plant interaction mediated by chemicals released in the environment, and significant advances have been made in recent years by using creative experimental design, sophisticated chemical analyses and careful data interpretation. Advances have been made in fundamental understanding of the process as well as its application in agriculture, forestry, rangeland and aquatic ecosystem management. There is no denying that allelopathy plays a prominent role in ecology and evolution of plant communities. However, its pervasive interacting nature intrigues us as well as challenges us as scientists to dig deeper into the understanding of its mechanism of action. Working on this challenge will lead to new discovery that will keep us excited to learn more and gain a better understanding of the phenomenon. Equipped with this new knowledge and understanding, we should be able to solve many difficult environmental problems of our time.

2.1 Introduction

The idea that plants affect neighboring plants by releasing chemicals in the environment has been known since c. 370 BC (Willis 1985, 1997). Greeks and Romans used this knowledge in practicing agriculture as early as 64 AD. The antagonistic effects of certain tree species such as walnut tree (*Juglan* spp.) on understory plants and nearby crops were also known to humans centuries ago (Rizvi and Rizvi 1992; Willis 2000, 2002, 2004). Poor yield of repeated cultivation of certain crops and fruits due to so-called 'soil sickness' has been known and investigated since the beginning of horticulture. Although this form of plant–plant interference has been known for quite some time, it is only recently (1937) that the Austrian plant physiologist, Hans Molisch, gave it a formal name, allelopathy (Molisch 1937, 2001). During its long history, allelopathy was perceived as a donor–receiver phenomenon where one plant releases chemicals that affect the growth of the neighbouring plants mostly in agri-

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cultural and horticultural settings. The idea of allelopathy as an ecological phenomenon structuring plant communities is rather recent. Whittaker and Feeny (1970) recognized the role of allelochemicals in plant community organization. In the 1960s and 1970s through a series of field and complimentary laboratory studies several authors provided data showing that certain plants can influence their neighbouring community of plants directly by releasing allelochemicals and indirectly by affecting the activity of rhizosphere microbes (Muller 1966, 1969; Muller et al. 1964; Whittaker and Feeny 1970; Rice 1964, 1965). However, their assertion was met with skepticism following a demonstration that bare ground created around allopathic shrubs can be invaded by plants after removal of herbivores (Bartholomew 1970) generating the famous criticism from J.L. Harper who described allelopathy as a complex 'undeniably natural phenomenon' but 'nearly impossible to prove' (Harper 1975). The following four decades experienced a great deal of skepticism by the general plant ecologists in accepting almost any results suggesting the presence of allelopathy as a viable explanation of plant–plant interaction principally on the ground that effects of other factors have not been removed in demonstrating allelopathy. Unreasonable 'burden of proof' was imposed on the researchers proposing allelopathy as a plant–plant interference mechanism (Williamson 1990; Willis 1985). By the end of the last century several authors suggested that allelopathy can not only affect neighbouring plants and influence plant community structuring, but it can also induce a broader ecosystem level change when it coincides with disturbance (Zackrisson et al. 1997; Wardle et al. 1997; Mallik 1995). In a well-written review, Wardle et al. (1998) argued that the concept of allelopathy can be applied more effectively at the ecosystem-level rather than at population level of resolution. Recently, several authors published convincing evidence of allelopathy as ecological mechanisms of exotic plant invasion through a series of well designed experiments and sophisticated chemical analyses (Bais et al. 2002, 2003; Callaway and Aschehoug 2000; Vivanco et al. 2004). These authors also argued ecosystem-level vegetation change following exotic invasion.

In my address today I would like do three things: first, briefly review the evolution of the concept of allelopathy from individual to population to ecosystem-level perspective, highlight the interplay of ecosystem disturbance and plants reproductive strategy in bringing about ecosystem-level changes; secondly, impress upon the need for fundamental research to discover the mechanisms of allelopathic interactions not only to solidify the scientific basis of the discipline but also use this knowledge to develop new methods for sustainable land management strategy. I shall end my contribution by highlighting some current challenges, opportunities and future directions in allelopathy research.

2.2 Evolution of the Concept of Allelopathy

Early observations and experimentations in allelopathy were based on the concept that allelopathic donor plants release allelochemicals (from root exudates, volatiles from above ground components or decaying plant litter) in the environment that interferes with the growth of nearby plants. From this, the donor plants would gain competitive advantage, out-compete their neighbors and bring about individual and population level changes. Molisch (1937) demonstrated, with simple laboratory experiments, that toxic volatile (ethylene) from plant (apple) can affect the growth (wilting, bud and root inhibition of stem cutting) of other plants. From this he cautiously speculated that chemicals of plant origin (allelochemicals) have potential for bringing about population level change by affecting the growth of neighbouring plants. He named this phenomenon allelopathy and became the father of allelopathy. Unfortunately, we inherited the excessive use of the term 'potential' and a habit of using simple experiments to draw far reaching conclusions without giving much thought to the complexity of other ecological interactions in the natural world. The result has been an issue of credibility where most of the early work on allelopathy has failed to withhold the rigor of scientific scrutiny (Willis 1985; Romeo 2000; Mallik 2000).

2.3 Disturbance and Ecosystem-Level Change

Type, frequency and intensity of disturbance by interacting with plants' regeneration strategies and allelopathic properties can dictate the direction of succession following disturbance. For example, forest canopy removal by clearcutting and non-sever fire can stimulate certain ericaceous plants with allelopathic property to dominate the post disturbance landscape and resist tree invasion, transforming forests into ericaceous heath (Mallik 2003; Zackrisson and Nilsson 1992). Zackrisson et al. (1996) published field evidence showing that natural fires perform key ecological functions in maintaining conifer forests of northern Sweden by removing competition and allelopathy from the ericaceous plant, *Empetrum harmaphroditum*, by adsorbing allelochemicals (Batatasin III) in charcoal, removing phenol rich humus by thermal combustion and creating a favorable seedbed for conifers. Fire suppression and clearcutting on the other hand promote understory ericaceous growth causing an ecosystem level vegetation change where *Empetrum* allelopathy plays a significant role in inhibiting germination and seedling growth of tree species (Nilsson 1992).

In the case of the nutrient poor black spruce-*Kalmia* ecosystems in eastern Canada, the prominent role of competition in structuring plant community following disturbance can be preempted by allelopathy and seedbed limitation (Bloom 2001; Mallik and Roberts 1994). Canopy removal by clearcutting and the absence of high intensity natural fires stimulate vegetative growth of *Kalmia*, transforming forest into ericaceous heath, which resists tree colonization. High *Kalmia* cover, in the absence of a canopy tree, produces large amounts of litter rich in polyphenols, which can induce long-term physical and chemical changes in the soil creating an alternate persistent vegetation state (Bloom and Mallik 2004). The 'afterlife effects' (Bergelson 1990; Wardle et al. 1997) of the ericaceous litter makes the soil more acidic; their phenolic allelochemicals bind N in protein–phenol complexes and the habitat becomes further deficient in available N (Bending and Read 1996a,b; Mallik, 2001). In the presence of a large array of phenolic acids, metallic cations such as Fe, Al, Ca, Zn, Mn etc. precipitate to the lower soil horizon and form hard iron pans altering the soil–plant–water relation (Inderjit and Mallik 1996). With rapid build up of acidic humus and a high rate of paludification, occupancy of the ericaceous community brings about long-term change in the habitat that is less and less suitable for conifer regeneration (Gimingham 1960, 1972; Damman 1971, 1975; Meades 1983; Bradshaw and Zackrisson, 1990; Prescott et al. 1996). Following Jones et al. (1994) and Lawton and Jones (1995), one can argue that this persistent vegetation state is a result of ecological engineering effects brought about by the combined effect of polyphenol rich *Kalmia* humus with allelopathic property, as well as competition from its aggressive vegetative regeneration strategy (Mallik 1993, 1994; Zhu and Mallik 1994).

Wardle et al. (1998) reported another example of site preemption by an invading weed nodding thistle (*Carduus nutans*) in New Zealand pastures dominated by perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*). In this case, through aggressive seed regeneration in small pasture gaps (5 cm diameter) followed by rapid vegetative growth of its rosette leaves the invading species cause expansion of the invaded patch (up to 1 m diameter). The thick rosette leaves of *C. nutans* undergo very rapid decomposition producing a strong allelopathic effect on *T. repens*, completely displacing it from the patch. Nodulation and nitrogen fixation of white clover is seriously inhibited by the leaf decomposition product of *C. nutans* leaving the patch relatively nutrient poor compared to the adjacent area. The authors were able to discount other ecological effects such as competition for light, nutrients and herbivory from this interaction to demonstrate the over riding effect of allelopathy in displacing *T. repens*. Wardle et al. (1998) suggested that by inhibition of nitrogen fixation in the presence of leaf decomposing allelochemicals, *C. nutans* can induce long-term nitrogen decline in such a pasture ecosystem.

Allelopathy induced ecosystem-level effects of exotic invasive plants have been reported by several authors (Hierro and Callaway 2003; Vivanco et al. 2004). Through a series of greenhouse, field and laboratory experiments these authors demonstrated convincingly that root exudates of *Centaurea diffusa* and *Centaurea maculosa*, natives of Eurasia and exotics noxious to Palouse and intermountain prairies of North America, can not only directly affect the root growth of the North American native plants but also their rhizosphere microbes (Callaway and Aschehoug 2000; Bais et al. 2002, 2003). Not only do these exotic plants bring with them novel allelochemicals that adversely affect germination and growth of native plants, but the chemicals stimulate the synthesis of allelochemicals by their rhizospheric biota (Callaway 2002; Ridenour and Callaway 2001). Extensive use of these exotic chemical weapons, in conjunction with unique seed regenerating strategy and perennial habit of the invasive plants, brings about ecosystem-level changes creating monospecific stands and change the chemistry and biophysical properties of soil (Callaway and Ridenour 2004).

2.4 Establishing the Scientific Basis of Allelopathy

This is the theme of the 2005 congress. It is appropriate and timely. By reading the history of allelopathy one can easily conclude that in addition to some useful discoveries, the past was replete with observations, hypotheses, experiments and conclusions that were often simplistic and scientifically unfounded (Willis 1985, 2004). As a result, the main stream ecologists practically ignored research on allelopathy, essentially claiming that the influence of other major factors such as resource competition, soil chemical and biological properties have not been considered and successfully eliminated to demonstrate the effect of allelopathy. The critiques had some valid reasons to be skeptical, but the demand for unequivocal proof often becomes too much of a burden to bear (Willis 1985; Williamson 1990). Unfortunately, the trend of making broad and generalized conclusions unsupported by data continues, despite repeated cautions and explanations of the complexity of the phenomenon that call for careful and logical experiments and responsible data interpretations (Romeo 2000; Inderjit and Weston 2000; Inderjit and Callaway 2003). Remarkable progress has been made in the last four decades. Demonstration of allelopathy mechanisms that were once considered impossible (Harper 1975) have been achieved by creative experimentation and use of advanced biomolecular analytical techniques (Bais et al. 2003; Vivanco et al. 2004). In order to establish the discipline (allelopathy) on a solid scientific footing we must strive to demonstrate the mechanism of allelopathy in explaining plant to plant interactions and community structuring. Because of its interacting nature, any discovery in allelopathy will require interdisciplinary collaboration involving ecophysiologists, biochemists, molecular biologists, microbiologists, soil scientists and ecosystem ecologists. The nature of the research question will determine the type of collaboration required.

2.5 Current Status and Future Direction

2.5.1 Experimental Evidence of Allelopathy: A Nagging Preoccupation

I eluded to this issue in the earlier section. In general the most important challenge for allelopathy researchers has been to demonstrate the effects of allelopathy separating it from other associated processes under field or experimental conditions. This ‘burden of proof’ placed upon the experimentalists makes them think critically before arriving at conclusions and in general this principle served modern science well. However, because of the interacting nature of allelopathy which readily crosses the boundary of many disciplines, it is often difficult to demonstrate allelopathy. Some suggested that it is so intimately associated with other biotic and abiotic process that it is impossible to separate allelopathic effects from other related processes under field conditions (Inderjit and Del Moral 1997). Indications of the presence of allelopathy are not enough. To be convinced, one has to prove it ‘beyond reasonable doubt’ by eliminating all other possibilities (Williamson 1990). Because of the complexity involved it is ‘nearly impossible’ to demonstrate allelopathy experimentally (Harper 1975). But progress has been made by using innovative experimental designs and sophisticated biochemical and molecular techniques. A recent paper by Bais et al. (2003) has done what thought to be impossible in Harper’s days in the 1970s. The article attracted the attention of critics and skeptics alike generating a headline in Science ‘making allelopathy respectable’ (Fitter 2003). No doubt it was an excellent piece of scientific work, clever and convincing with a detailed investigation where intricate ecophysiological links have been traced and uncovered experimentally. The International Allelopathy Society (IAS) has rightfully awarded this year’s Grodzinski award to the authors for this publication. It is practically unheard of for allelopathy researchers to get editorial praise in *Science*. However, while giving a full recognition for the work in question the headline also reminds us of the skepticism as if prior to this paper allelopathy research was not respectable. Novel approaches in demonstrating allelopathy or separating allelopathy from competition have been published earlier and one can say that they were pretty respectable (Nilsson 1994; Weidenhamer et al 1989; Callaway and Aschehoug 2000; Mattner and Parbery 2001; Ridenour and Callaway 2001). To be acceptable not all experiments have to demonstrate ‘proof’ by isolating specific allelochemical(s) or discovering allelopathy mechanisms. Arguments can be made by experimentally eliminating associated factors (see for example Mattner and Parbery 2001). Besides, there is also a place for measured speculation based on the data trends and logical deductions.

2.5.2 Methodological Challenges

This has been a serious issue for most allelopathy experiments. Doing allelopathy experiments is not a trivial matter. Questions must be clear and logical. To be relevant, experimental design must reflect the ecosystem condition as best as possible (Inderjit and Callaway 2003). Elegance in experiment can come from clever and simple designs (e.g. Nilsson 1994; Callaway and Aschehoug 2000) as well as complicated design and thorough analyses, which often requires collaboration from several related disciplines (Bais et al. 2003; Vivanco et al. 2004). In either case, clearly identifying good research question(s) is crucial. Since allelochemicals are involved in the process, good knowledge in chemistry or collaboration with natural product chemists is often necessary. Simple bioassay with unrelated plants and artificial media can be of limited use in answering any mechanistic or applied questions. This does not mean that all allelopathy studies require a sophisticated chemistry laboratory. Manipulation experiments can be performed in the field, greenhouse or in lab that can answer both fundamental and applied land management questions (see Mattner and Parbery 2001). But the habit of making generalized process based comments based on quick and unrealistic bioassay must stop. The history of agriculture is replete with examples of ancient traditional biological and cultural methods of crop protection. Hints can be obtained from these traditional cultural practices, some of which can be explained by allelopathy (Anaya 1999). Refinement of many traditional techniques of crop rotation, multiple cropping, mulching, cover cropping, green manuring and microbial inoculation can enhance crop productivity by reducing or in some cases eliminating the use of agrochemicals resulting in improvement of environmental quality. Over the last four decades many allelopathy bioassays have been conducted. The time has come to use these results to demonstrate the application of this knowledge by conducting statistically designed large-scale field trials.

2.5.3 Open-Ended Definition, Seeking Boundary of the Discipline

There have been concerns expressed as to the limits and bounds of allelopathy. The widely used definition of Rice (1984) ‘any direct and indirect effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment’ is viewed as all-encompassing and lacks any boundary. The definition of the IAS, ‘any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influence the growth and development of biological and agricultural systems’ (IAS 1996). This definition also suffers from being too broad and had limited use since its publication. Many secondary metabolites of plants in the rhizosphere include sugar, simple polysaccharides, amino acids and other organic acids are not all allelopathic (Bertin et al. 2003). In describing algal allelopathy Inderjit and Dakshini (1994) defined allelopathy as ‘a phenomenon where allomones contributed by the algae can affect: (1) other algae in its vicinity, (2) its own growth, (3) microbes associated with it, (4) higher plants in its vicinity, and (5) accumulation and availability of nutrient iones which influences the distribution, growth and establishment of other algae, microorganisms, and plants’. Unlike others, this is a specific but wordy definition that highlights autotoxicity. Several others have described allelopathy as: (i) ‘allelopathy is an interference mechanism by which plants release chemicals that affect other plants’ (Wardle et al. 1998), (ii) ‘allelopathy is the negative effect of chemicals released by one plant species on the growth and reproduction of another’ (Inderjit and Callaway 2003), (iii) ‘allelopathy is the release of extracellular compounds that inhibit the growth of other microorganisms’ (Suikkanen et al. 2004), (iv) ‘suppression of neighboring plant growth by the release of toxic compounds’ (Fitter 2003), (v) ‘release of chemical compounds by an invader that have harmful effects on members of the recipient plant community’ (Hierro and Callaway 2003) and (vi) ‘the chemical suppression of competing plant species’ (Vivanco et al. 2004). One thing seems to be common in all these definitions except Rice (1984) is that they all refer allelopathy to negative effects as did the authors writing in the 1950s and 1960s (Muller et al. 1964; Muller 1966, 1969; Rice 1974). Examples of stimulatory effect of allelopathy are rare and usually associated with low concentration effects of the compounds (Rice 1984).

The word allelopathy does not appear in popular reference such as the new Oxford Encyclopedic Dictionary, despite many thousands of peer-reviewed publications in the English language. Where it does appear, such as in the Oxford English Dictionary *Additions Series*, the Webster’s Dictionary and the Encyclopedia Britannica, allelopathy refers to chemicals released by plants having strictly negative effects on neighbouring plants (Willis 2004). Often authors define allelopathy based on their worldview from a very narrow to a very broad perspective. Inderjit (2001) suggested that allelopathy should refer only to the inhibitory effects of allelochemicals. One of the main reasons for having this difficulty with the definition is because the allelopathic phenomenon is linked directly and indirectly with a diversity of physical, chemical and biological processes involving a large array of compounds and their precursors. However, no matter how complicated it is we must have an acceptable definition indicating the limits and boundary of the discipline. With respect to specialization and worldview we must consider the rich disciplinary diversity of the field as strength rather than an obstacle because complicated research questions in allelopathy can only be answered through interdisciplinary research. As the mechanism of allelopathy becomes more and more clear to us it will be easier to define. I can think of three things requiring attention in redefining allelopathy (i) mechanism, (ii) evolutionary significance (i.e. the need for plants to have this property) and (iii) the outcome (effects on neighbors ecosystems).

2.6 Future Direction

2.6.1 Self-Criticism and Good Science

In order to think about future directions we must reflect on how we are doing now, what is working and what is not working. In my view, we are doing well and lately research in allelopathy has made quite a breakthrough, at least in the sense that the scientific community is becoming more receptive to the idea that it is worthwhile to pursue research in allelopathy. This has been possible only because of good science published in influential journals. We have to do more of this to make an impact. It is a challenging but necessary field of research that can keep us intrigued because of its complexity and applications in sustainable resource management. What has not worked in the past and will not work in the future is a half-hearted endeavor. Allelopathy research without clear and logical question(s), unjustifiable methodology, poor data and unreasonable conclusions has been counter productive. There is a tremendous opportunity for allelopathy researchers to contribute to the achievement of sustainable management of natural resources be it forestry, agriculture, horticulture, grassland, rangeland, parks, ecological reserves and conservation areas. I have already mentioned that for too long we have been preoccupied to find ‘proof’ of allelopathy. The time has come to take the next step. By working collaboratively with chemists, agronomists, ecologists, hydrologists, bio-statisticians and most of all with the farmers, foresters and aquaculturalists, we must try to solve the ecological problems at hand by using the knowledge of allelopathy. It is only then we can gain respect for our science and our profession.

2.6.2 Allelochemicals as Weed Control Agents, Signaling Molecules and Genetic Manipulation

Birkett et al. (2001) made a literature review asking the question whether allelopathy offers real promise for practical weed management. There are several different approaches to weed control, most popular being total elimination of weeds by chemical herbicides widely practiced in industrial agriculture. There are potent allelochemicals in plants (Nimbal et al. 1996; Czarnota 2001) but the chance of finding allelochemicals that can be used as industrial bioherbicides for successful weed control is not good (Duke et al. 2001). However, microbially synthesized herbicide such as bialophos has been in the market for several years showing success in weed control in agriculture and forestry (Jobidon 1991). The second approach is using allelopathic plants as mulch, cover crop, row crop etc that take advantage of not just the chemicals inhibiting seed germination and growth of crops but its biomass which physically suppress weed growth (Mattice et al. 1998; Moyer et al. 2000). This method does not aim for complete eradication of weeds by chemicals but rather allows coexistence of competing plants with much reduced vigor. The biomass added to the soil in this practice incorporates organic matter to the rhizosphere which influences the soil microbial ecology and nutrient conditions. Traditional agriculture has been using this approach of weed control and there is room for further improvement through research.

Following extensive lab and field trials several allelopathic rice varieties have been selected (Dilday et al. 1991) and although it promises to be challenging we are steps closer in transferring allelopathic property in rice by plant breeding (Olofsdotter 2001a,b; Olofsdotter et al. 2002). Genetically modified rice with insect resistance is currently at pre-commercial field trial stage in China (Huang et al. 2005), Golden rice 2 has been genetically engineered by incorporating certain enzymes (psy) from maize. This has resulted in a dramatic increase in Beta-carotine (pro-vitamin A). Use of this rice can reduce vitamin A deficiency, a common and very serious problem encountered in a large population in Asia where rice is the staple food (Paine et al. 2005). Research is underway in identifying the signal compounds in plants that can synthesize chemicals used for defense for insects and pests. If gene transfer for insect resistance, pro-vitamin A or signaling compounds from plant to plant is possible then transfer of allelopathic genes for weed control is also possible. Success in this area will definitely reduce and in some cases eliminate the use of herbicides and pesticides. There are exciting research and development opportunities in these emerging fields. However, as in many GM organisms the risk of long-term adverse effects of such genetic manipulations on the environment and human health must be assessed.

In conclusion I would like to emphasize three points: (i) in allelopathy research we must keep a dual focus, (a) fundamental mechanistic and (b) applied problem solving, (ii) we must enhance interdisciplinary collaboration and (iii) we must strive for effective communication by publishing results in peer reviewed journals, organizing workshops and conferences where we learn from each other through discussions. The Fourth World Congress on Allelopathy provides such an excellent opportunity and we very much appreciate the hard work of the local organizing committee under the leadership of Professor Jim Pratley.

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3. Allelopathy in Chinese Ancient and Modern Agriculture

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Abstract. Over the last 2000 years, allelopathy has been widely reported in ancient Chinese literature with a focus on Xiang Sheng Xiang Ke, which is the beneficial and inhibitory interference between plants. Fan Sheng Zhi Shu (first century BC) is the earliest Chinese agricultural book available to describe plant allelopathy. This chapter reviews many records about allelopathy and its application in agriculture in the ancient Chinese agricultural books. These descriptions in ancient books indicate that the knowledge about plant allelopathy was an important theory and guidance for crop interplanting and weed management in Chinese ancient agriculture. Modern allelopathy research in Mainland China started in 1981 and grew rapidly after 2000. Allelopathy of staple crops such as rice and wheat, invasive alien plant species and some noxious weeds are the focus of allelopathy research in China. Considerable progress has been made in isolation and identification of allelochemicals, molecular mechanisms, and modes of action since the 1990s. Future directions and prospects of allelopathy research are discussed in this chapter.

3.1 Introduction

Rice (1984) defined allelopathy as the effect of one plant (including microorganisms) on the growth of another plant through the release of chemical compounds into the environment. Both beneficial and inhibitory effects are included in this definition. Allelopathy plays an important role in plant interactions and in structuring plant communities; plants have various complicated relations with their neighbors, including competition, inhibition, stimulation and interdependence. Some plants grow with many other species together, but some grow in a monoculture community and prevent other plants from growing in the nearby environment. Overall influence of one plant on another in the vicinity is called interference, which includes competition and allelopathy (Muller 1969; Rice 1974). Competition refers to the ability of a plant to deplete a limited resource from the environment, resulting in reduced fitness of competing plants. Allelopathy refers to the interference caused by one plant to another through release of chemicals. All parts of plants including roots, stems, leaves, flowers, fruits and seeds could release chemicals into the environment. Plant residues also release allelochemicals after microbial degradation. Plants use these chemicals to interfere their neighbor's growth.

China has the largest population in the world and its arable land per capita is very small. The multi-cropping index of arable land is high. Interplanting has a long history and is still being used in agriculture and forests. Herbicide application is not very common. Allelopathic interactions between plants may play a vital role in weed management and cropping system selection. Allelopathy exists between crops and weeds, previous and consecutive crops, or interplanted crops. Allelopathy research not only leads to a better understanding of natural community structure and succession, but also provides an alternative approach for weed management, sustainable development of agriculture and a new source of natural herbicides (Rice 1984; Duke et al. 2002; Reigosa et al. 2006). This chapter describes allelopathy records in ancient Chinese literatures and modern allelopathy research in Mainland China.

3.2 Allelopathy in Ancient China

Chinese agriculture has more than 7000 years of history (Yu 1987). Herbicides were not applied at all until the 1980s. Weeds in crops were controlled successfully by tilling, hand weeding, interplanting, crop mulching and crop rotation. In China natural products have been used for medicinal purposes for over four thousand of years (Leung 1990) and the earliest record of Chinese herbal medicine was between 1065 and 771 BC in the Wu Shi Er Bing Fang (prescriptions for 52 diseases) (Sun et al. 1986). The term allelopathy was first coined by Molisch in 1937. However, the ancient Chinese observed and recorded the phenomenon of plant chemical interference (although they did not call it allelopathy) more than 2000 years ago and applied the knowledge for increasing crop yield, controlling weeds, diseases and pests (Zhou 1998). As such for over 2000 years, allelopathy has been reported in many ancient Chinese books with respect to plant Xiang Sheng Xiang Ke (beneficial and inhibitory interference).

Fan Sheng Zhi Shu (also called *Fan Sheng Chih Shu*, first century BC) is the earliest Chinese agricultural book available to describe plant allelopathy. The author Fan Sheng Zhi claimed that cucurbit and leek (*Allium porrum*) could be interplanted to reduce the disease of cucurbit, because leek could produce special substances to inhibit pathogens of cucurbit (Shi 1963). At that time, the *Allium* plant was recognized not only as a spice but also as a substance that exerts a control on microbial pathogens. Recent publications indicate that garlic extract has broad-spectrum antimicrobial activity against many genera of bacteria and fungi (Iwalokun et al. 2004). The active component (allicin) has been isolated and characterized (Cavallito et al. 1944). The author also suggested that cucurbit plants could be interplanted with red bean (*Phaseolus vulgaris*). *Wu Li Lun* written by Quan Yang (third century AD) states that sesame inhibits weed growth (Zhou 1998).

Qi Min Yao Shu (also called Ch'i Min Yao Shu, sixth century AD) means 'essential ways of living for the common people'. It is the best-preserved and most influential agricultural work from ancient China. The book laid a good foundation for intercropping, both theoretically and practically. After a long time of war, Bei Wei (Northern China) paid special attention to agricultural development. At that time, intercropping and rotation were quite common practices in China. The author Sixie Jia, who was a governor of the District Kaoyany in the years from AD 533 to 544, systemically summarized the experience of farmers with strong emphasis on intercropping and rotation (Shi 1957; Shi 1962). The book gives a detailed list of crops that were good combinations for interplanting, as well as those that were not. The chapter entitled ‘Planting of Mulberry’ describes that root exudates of mungbean and *Vigna angularis* stimulates the growth of mulberry (*Morus* sp.) (Miao 1982). The two leguminous crops were good partners of mulberry for interplanting. The chapter entitled ‘Planting of Cucurbit’ indicates that cucurbit and soybean could improve each other’s growth and increase each other’s yields; the bleeding sap of beans stimulates seed germination of cucurbit plants and fertilizes the soil. The chapter ‘Planting of Hemp’ warns that cannabis (*Cannabis sativa*) and soybean (*Glycine max*) cannot grow together because they hate each other. The cannabis and sesame plants can be used as a crop fence in gardens, because they are poisonous and can prevent animal feeding. *Cannabis sativa* contains many alkaloids (Feraly and Turner 1975) and Singh and Thapar (2003) confirmed that it has allelopathic potentials. Sesame (*Sesamum indicum*) was used as a pioneer crop in wastelands since roots of sesame plants are poisonous to weeds. Its allelopathic potentials were demonstrated by Duary (2002). The proverb: “No weed grows in wilderness one year after sesame grows” is still widespread.

Fen Men Suo Sui Lu (twelve century AD) states that planting chive (*Allium schoenoprasum*), garlic (*A. sativum*) or leek (*A. porrum*) around flower plants could effectively protect flowers against attack by musk deer (*Moschus moschiferus*) (Wen 1962). *Allium* plants play an important role not only in Chinese traditional medicine but also in ecological control of pathogenic microorganisms in agriculture. Many Chinese people still eat garlic to prevent disease. The book also claims that no plant could grow underneath *Cinnamomum cassia* Presl. as it is known to kill its neighbors. When certain plants are planted under this tree they tend to yellow, wilt, and die. If a piece of Cinnamomum wood is nailed on other trees, the tree would die. The book also describes that tung oil can kill lotus (*Nelumbo nucifera*) plants (Wen 1962).

In Yuan Dynasty, Zhong Yi Bi Yong Bu Yi recorded that spinas of *Gleditsia sinensis* Lam. prevented spread of bamboo roots (Zhou 1998). Chen Fu Nong Shu (1149) described that root exudates and leaf leachates of ramie (*Boehmeria nivea*) stimulate growth of mulberry. Root distribution of the two crops is different (Miao 1981). They are a good combination for intercropping. Nong Sang Ji Yao (1273) claimed that interplanting mungbean, sesame, potato and black soy beans stimulated the growth of mulberry and increased leaf production by 20–30% (Shi 1982). At that time there was a widespread proverb: “mulberry stimulates proso millet (*Panicum miliaceum*), and proso millet stimulates mulberry”. The book also states that mulberry and corn cannot grow together.

The development of ancient Chinese agriculture reached its zenith in the Ming dynasty (1368–1644). During this period more than 130 works on agriculture were written and published (Wang 1989). Bian Min Tu Cuan (1493) summarized the experience in the success of plantations in wilderness (Kuang 1959). After burning and ploughing, one year of sesame planting could successfully control weeds because the root exudates of sesame would kill the weed roots remaining in the soil. Cha Jie (1609) explained that tea tree should be planted in the vicinity of fragrant plants and that malodorous plants in the vicinity could affect the tea quality (Zhou, 1998). Qun Fang Pu (1621) described that cinnamomi stimulated the growth of yam (*Colocasia* *formosanum*), cassava (*Manihot esculenta*) and mountain ginger (*Alpinia pumila*) when interplanted in young cinnamomi forest (Yi 1985). No grass grew underneath cinnamomi plants. It has been confirmed that cinnamomi contained many compounds with antifungal activity (Giordani et al. 2001). *Nong Zhen Quan Shu* (AD 1628), written by Guang-qi Xu, was the best reflection of agricultural achievements in the Ming dynasty (Shi 1979). The book states that beans underneath a chestnut canopy not only fertilizes the soils but also controls the weeds.

During the Qing Dynasty, Hua Jing (1668) stated that Chinese cinnamon (*Cinnamomum cassia*) inhibited the growth of grasses and that liquorice stimulated the growth of flowers; mulching with cinnamomi chippings effectively controlled weed growth (Chen and Yi 1962). Nong Pu Liu Shu (seventeen century AD) reported that water leachates and dew of sesame are inhibitory to the growth of other plants; plants that made contact with the dew of sesame would wilt and die. Weeds grew poorly in the second year of sesame growth, indicating that some inhibitory substances existed in water leachates and dew of sesame (Newsroom of China Agriculture Cyclopaedia 1995). The book warns that flowers and trees could not be planted in the vicinity of sesame. Zhi Fu Qi Shu (late seventeen century AD) and Yang Yu Yue Ling (1640) described similar records of sesame's ability to kill weeds and be used as pioneer plant species in wilderness (Zhou 1998). Zhi Fu Qi Shu recorded the appropriate approaches to developing agriculture in the wilderness: burning weeds, ploughing, planting sesame at the beginning, and then cultivating cereal crops. Planting sesame resulted in weed control and also rapid decomposition of root residues in soils (Newsroom of China Agriculture Cyclopaedia 1995).

Guangdong Xin Yu (late seventeen century AD) stated that sugarcane and banana were good rotation crops since they stimulated each other via root exudates and residues in the soils (Zhou 1998). San Nong Ji in Qing Dynasty claimed that Yegoma oil plant (*Perilla frutescens* (L.) Britton var. *crispa*) and *Perilla frutescens* (L.) Britton can be used as pioneer crops for weed control. Also during the Qing Dynasty, *Hu Zhou Fu Zhi* (1862–1874) written by Yuan-han Zong and Xue-jun Zhou, warned farmers not to plant mulberry with wheat and barley together (Newsroom of China Agriculture Cyclopaedia 1995).

3.3 Allelopathy in Modern China

3.3.1 1980s

Allelopathy research in China started in the 1980s. Approximately 10 research articles and 14 reviews were published in the 1980s. Zhang and He (1981) published the first scientific research article on allelopathy in China. Lousewort (*Pedicularis longiflora*) is a common annual weed in barley fields of the Qingzang Plateau. The weed severely inhibits the seed germination and seedling growth of barley. Water leachates of the plant completely inhibited the seed germination of barley, wheat and eight other plants at a concentration of 0.2 g ml^{-1} (dry weight of intact weeds). One component consisting of 6% of the total water leachates, completely inhibited wheat seed germination at 6.0 mg ml^{-1}; it damaged roots and inhibited cell division and elongation (Zhang and He 1981).

Xin et al. (1986) reported the in vitro allelopathic effects of tube-seedlings of *Salix siuzevii* on species such as *Betula platyphylla* and *B. costuata*. Root exudates and water leachates from in vitro seedlings of *S. siuzevii* inhibited root growth of the tested plants. Yang et al. (1987) first isolated an active allelochemical, harmaline, from harmal (*Peganum harmala*), a perennial grass in Northwest China that is used as green manure by local farmers. This compound inhibited seedling growth at high concentrations and stimulated seedling growth at low concentrations (Yang et al. 1987). Mang qi (*Dicranopteris dichotoma*) is a pioneer species and also an indicator of acidic soil. The plant has a strong competitive capability against other species in the Subtropics of China. The water leachates of *D. dichotoma* showed strong inhibitory effects in both laboratory and pot experiments on all nine tested species except for Chinese red pine (*Pinus massoniana*), another pioneer species in the Subtropics of China (Ye et al. 1987).

Water hyacinth (*Eichhornia crassipes*) has become the most troublesome invasive weed in aquatic ecosystems worldwide. Sun et al. (1988) demonstrated the allelopathic effects of water-hyacinth (*E. crassipes*) on algae. The eutrophic water from plant cultivation suppressed algal growth. The root exudates decreased the photosynthetic rate and root activities, and damaged the chlorophyll a of algae.

Li et al. (1987a) investigated the allelopathic effects of plant residues on rice, rape and wheat. Roots were more susceptible to residue allelochemicals compared to shoots and coleoptile growth, and rice was more resistant than wheat and rape. Li and Yu (1988) further isolated and identified allelochemicals from decomposed wheat residues and its soil. The p-hydroxybenzoic, ferulic, coumaric and vanilic acids were found in the residue extracts and soil. The activity of amylase and catalase of wheat and rice seedlings were remarkably inhibited, while the peroxidase activity was stimulated by these allelochemicals (Li and Yu 1989).

Ajania tenuifolia is one of the main invasive weeds in artificial grassland. Wild rye (*Elymus nutans*) is the preferred pasture grass in Haibei in Qingzang Plateau. A positive correlation was found between *A. tenuifolia* invasion and *E. nutans* degeneration (Zhang et al. 1989a). Further study showed that the exudates of *A. tenuifolia* inhibited the seed germination of *E. nutans*, while the exudates of *E. nutans* inhibited the seed germination of *A. tenuifolia* (Zhang et al. 1989b). The exudates of *E. nutans* during the seed germination also had autotoxic effects on germination when the sowing density was high enough (Zhang et al. 1989b).

In the 1980s, a few scientists wrote allelopathy reviews to introduce concepts, methodology, identify allopathic plant species, and describe new developments in the field of allelopathy. Qian et al. (1982a, b) introduced allelopathy and interference concepts, history, phenomena of allelopathy in natural and manipulated ecosystems, and its significance in agriculture and forestry. Su (1986) predicted the potent application of plant and microbial allelochemicals to the development of new herbicides. Li et al. (1987b) outlined the existence of allelopathy in many crops, weeds, trees and microbes. They also reviewed classes of allelochemicals and their potent (potential?) modes of action. Chen and Tang (1988) claimed that allelopathy is ubiquitous in the plant kingdom and plays a key role in constructing plant communities. Chen and Tang (1988) suggested the future direction of allelopathy research in China. Xu and Li (1988) listed many plants that have demonstrated allelopathic potentials. Li (1989) systemically introduced the new advances in the science of ecological biochemistry, including allelopathy, in the Chinese Journal of Ecology.

3.3.2 1990s

In the 1990s, allelopathy research in China developed rapidly. More than 60 research articles and 30 reviews were published in the 1990s. Using allelopathy as a key word, we found more than 82 allelopathy papers published in Chinese in the database of China Academic Journal Electronic Publishing House. Using Hua Gan Zuo Yong (the formal translation of allelopathy to Chinese since 1992) as a key word, we found 98 papers, of which more than 25 were published by the Institute of Tropical & Subtropical Ecology at South China Agricultural University. This department is lead by Shi Ming Luo, who trained a batch of graduates who systemically conducted allelopathy research in China at the beginning of the 1990s (Zeng 1991; Cao 1992; Deng et al. 1996; Wei et al. 1997; Zhou et al. 1997). Only a few of the papers were published outside China in English (Yu and Matsui 1997; Yu 1999). Allelopathic potentials of many plant species, especially invasive species, were investigated in China in the 1990s (Cao 1992; Cao and Luo 1996b; Wang and Zhu 1996a, b; Zeng and Li 1997). Isolation and identification of allelochemicals were the focus of allelopathy research at this stage (Luo et al. 1995; Zeng et al. 1996; Deng et al. 1996; Wang and Zhu 1996a, b, c; Wei et al. 1997). Shi Ming Luo and his colleagues did some pioneer work on allelopathic effects of invasive alien species in South China and concluded that allelopathy is an important mechanism leading to the wide distribution of invasive alien species in novel ranges (Zeng 1991; Zeng and Luo 1993; Luo et al. 1995; Wei et al. 1997; Luo 2005).

3.3.2.1 Allelopathy in Compositae Species

Allelopathy of goat weed (*Ageratum conyzoides*) and bur marigold (*Bidens pilosa*) was demonstrated by Zeng (1991) and Zeng and Luo (1993). Root exudates of *A. conyzoides* and *B. pilosa* also showed allelopathic potentials (Zeng and Luo 1996). Zeng et al. (1994) reported the allelopathic effects of root exudates and water leachates of Chinese Wedelia (*Wedelia chinensis*). Wang and Zhu (1996a, b) identified the allelopathic potentials, through water leaching, of ragweed (*Ambrosia artemisiifolia*) and *A. trifida*, two important invasive species of crops in Northern China e.g. rice, wheat, corn and soybean. Aqueous extracts of *Artemisia ordosica* strongly inhibited seedling growth of *Amaranthus tricolor*, *Orychopragmus violaceus*, *Raphanus sativus* and *Triticum aestivum* (Ma et al. 1999). Crofton weed (*Eupatorium adenophorum*) is an aggressive invasive exotic species in Yunnan province. The water leachates strongly inhibited root growth of corn (Zhang et al. 1993). Scanning electron microscopy showed that the leachates restrained the root cell development and destroyed the cells between the epidermis and cortex of the roots (Zhang et al. 1993).

3.3.2.2 Allelopathy of Wheat and its Straws

The allelopathy of wheat straws has attracted a lot of attention because wheat straws have been used for mulching for a long time. Yang and Li (1992) reported the allelopathic effect of wheat on cogon grass (*Imperata cylindrica*). Wheat straw contains water-soluble toxic substances and inhibited seed germination and seedling growth of corn (Zhang and Ma 1994). 4.5 tons of wheat straw per hectare yielded the best growth of corn seedlings (Zhang and Ma 1994). The mulched straw showed the strongest inhibition to corn seedlings 5 days after planting. Significant variation existed among different corn varieties in tolerating allelopathic effects of wheat straw mulch. Using resistant corn varieties could avoid the negative allelopathic effect of wheat straw mulch (Ma and Han 1993). The stubble of different varieties of wheat had different inhibitory effects on the growth of successive corn (Ma et al. 1995). This indicates that varietal variations of autotoxicity exist not only in living plants (Chung and Miller 1995), but also in plant residues. Water leachates of wheat decreased the germination of redroot pigweed *Amaranthus retroflexus* by 86% at 500 mg kg^{-1} (Li et al. 1996). Field tests showed that 79% of *Stellaria media* plants were killed by the extract of wheat at 100 mg m^{-2} (Li et al. 1996). Li et al. (1997) isolated a component from wheat glumes that showed strong phytotoxic effects on *I. cylindrica*.

3.3.2.3 Allelopathy in Trees

Jia et al. (1995) investigated the allelopathic potentials of Chinese pine (*Pinus tabulaeformis*), Liaotung Oak (*Quercus liaotungensis*) in a mixed forest. Leachates of their litter and surface soil inhibited the seedling growth of Chinese pine and the other tested plants. *Eucalyptus* is one of the most economically important tree species introduced from Australia; it is widely planted in Southern China (Wu 1991). The seedling growth of Mangium wattle (*Acacia mangium*), radish (*Raphanus sativum*), lettuce (*Lactuca sativa*), and lead tree (*Leucaena leucocephala*) was inhibited by the leaf volatiles and aqueous extracts of *Eucalyptus citriodora*, *E. urophylla* and *E. exserta* (Cao 1992; Cao and Luo 1996a; Zeng and Li 1997). Leaf extracts of *Eucalyptus urophylla* inhibited the root initiation, root number and root length of hypocotyl cuttings of mungbean (*Phaseolus aureus*) and pea (*Pisum sativum*) (Huang et al. 1997), and also inhibited the seed germination of India mustard (*Brassica juncea*) and wild cabbage (*Brassica oleracea* var. *caulorapa*) (Huang et al. 1997).

Trees and crops grow together in agroforestry. However, Zhu and Wang (1997) found potent allelopathic effects from root leachates of apple (*Malus pumila*), Carolina Poplar (*Populus canadensis*) and other trees on wheat growth. Tea tree (*Camellia sinensis*), Chinese fir (*Cunninghamia lanceolata*) and Horsetail wood (*Casuarnia equisetifolia*) show autotoxicity. The main allelochemicals in tea plants, e.g. polyphenols and caffeine, inhibited the growth and seed germination of old tea tree (Cao and Luo 1996b). Water leachates from branchlets of Horsetail wood strongly inhibited the root growth of its own seedlings (Deng et al. 1996). Extracts of the soil collected from the second and the third rotation of Chinese fir replant woodlands significantly reduced the growth of Chinese fir seedlings (Zhang 1993) and influenced microbial activities and nitrogen mineralization dynamics in the soil (Zhang 1997).

3.3.2.4 Allelochemical Isolation and Identification

Waterhyacinth has an allelopathic effect on algae (Sun et al. 1988). Three compounds with anti-algal activities were isolated and identified as N-phenyl-2-naphthylamine, linoleic acid and glycerol-1,9-12(ZZ)-octadecadienoic ester (Yang et al. 1992). N-phenyl-2-naphthylamine was released into the culture media from the roots of waterhyacinth and had stronger anti-algal effects than $CuSO_4$, a common algaecide. Zeng et al. (1996) isolated and identified oxidoisotrilobolide-6-O-isobutyrate and trilobolide-6-O-isobutyrate from *W. chinensis*. The two compounds showed strong inhibition to the seedling growth of barnyardgrass, radish and lettuce. Allelochemicals of Horsetail wood (*C. equisetifolia*) were isolated and identified as kaempferol-3-α-rhamnoside, quercetin-3-α-arabinoside and luteolin-3',4'-dimethoxy-7-β-rhamnoside (Deng et al. 1996). The three compounds significantly inhibited the root growth of *C. equisetifolia* at the concentrations of 100 $\mu g\ mL^{-1}$ or higher. Luteolin-3',4'-dimethoxy-7-β-rhamnoside significantly inhibited the root growth at a lower concentration of 25 $\mu g\ mL^{-1}$. The α-pinent, β-pinene, cineole, camphene, spanthueol were identified as potent allelochemicals in *A. artemisiifolia* (Wang and Zhu 1996a). Volatiles in *A. trifida* plants include camphor, camphene, α-pinene, β-pinene, cineole, decane, nonane, hornylene, germacrene D, limonene and myrcene (Wang and Zhu 1996b, c). Ageratochromene (6,7-dimethoxy-2, 2-dimethylchromene) was identified as the main allelochemical in Tropic Ageratum (*A. conyzoides*) (Luo et al. 1995; Wei et al. 1997). GC/MS analysis showed that the main compounds in essential oil of *A. ordosica* are α-pinene, β-pinene, sabinene, capillene and nerolidol (Yu et al. 1996). Pisolactone isolated from fruitbodies of *Pisolithus tinctorius* significantly inhibited the seedling growth of *Echinochloa crus-gall* at 100 $\mu g\ ml^{-1}$ (Zeng et al. 1999).

3.3.3 After 2000

Allelopathy research in China has been growing dramatically since 2000 (Figs. 1–2). Using allelopathy as a key word, more than 290 allelopathy papers were found in Chinese in the Database of China Academic Journal Electronic Publishing House; using Kua Gan Zui Yong as a keyword, 459 papers were found. Many allelopathy research papers have been published outside of China in international journals such as the Journal of Chemical Ecology (Bi et al. 2007; Huang et al. 2003; Zeng and Mallik 2006), Agronomy Journal (Ma et al. 2004; Zeng et al. 2001a, b), Plant and Soil (Ye et al. 2004; Yu et al. 2000), Allelopathy Journal (Ma 2003; Zuo et al. 2005a), and Plant Growth Regulation (He et al. 2004). Chinese journals, including the Chinese Journal of Applied Ecology, Acta Ecologica Sinica, Acta Phytophysiologica Sinica and the Chinese Journal of Ecology, are the main journals publishing allelopathy work. Although all of these journals are published in Chinese, they provide English abstracts and references. There were more research articles published in between 2005 and 2006 than in the decade of the 1990s. Most studies are involved in allelopathy of crops e.g. rice and wheat (Lin 2005). Allelopathy of invasive plants has been widely investigated (Luo 2005). Molecular mechanisms involved in allelopathy have become the focus of allelopathy research since 2000 (Zeng et al. 2003; Xu et al. 2003b; He et al. 2005d; Bi et al. 2007). Modes of action of allelochemicals were determined (Lin et al. 2000; Zeng et al. 2001b; Nie et al. 2002; Yang et al. 2004). Mathematical modeling was employed to simulate the role of allelopathy in alien plant invasion and species interactions by students of Shi Ming Luo's (Liu et al. 2005; Liu et al. 2006a, b).

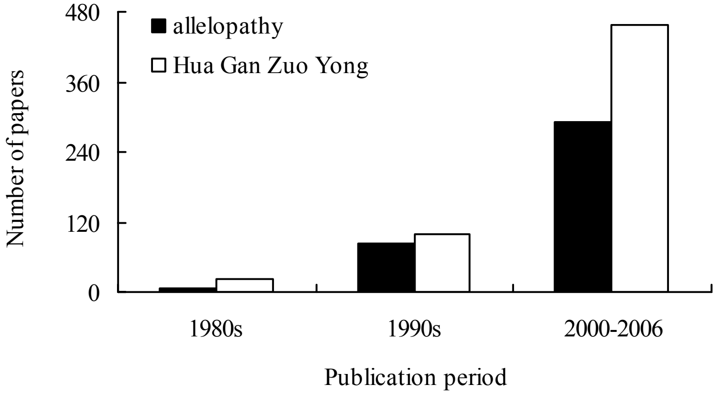

Fig. 1. Journal publications on allelopathy in Chinese during 1980–2006. Values were obtained from the Database of China Academic Journal Electronic Publishing House using allelopathy or Hua Gan Zuo Yong (formal Chinese translation of allelopathy) as key words on 27 Jan. 2007. Data for 2006 are incomplete at the statistical time point

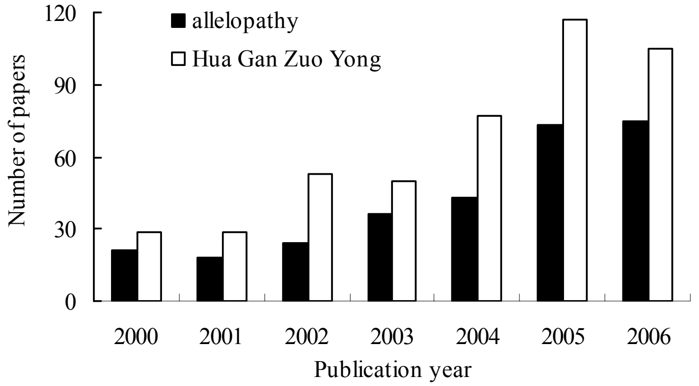

Fig. 2. Journal publications on allelopathy in Chinese during 2000–2006. Values were obtained from the Database of China Academic Journal Electronic Publishing House using allelopathy or Hua Gan Zuo Yong (formal Chinese translation of allelopathy) as key words on 27 Jan. 2007. Data for 2006 are incomplete at statistical time point

3.3.3.1 Rice Allelopathy

Rice (*Oryza sativa* L.) is the most important crop in China and many other countries in Southeast Asia. Over the last two decades, there is an increasing interest in rice allelopathy in China (see review by Lin et al. 2006; He et al. 2002a; Lin 2005; Zeng et al. 2003; Xu et al. 2003a; Xu et al. 2004), Japan (Kato-Noguchi 2004), India (Oudhia and Tripathi 1999), Korea (Kim and Shin 1998) and the International Rice Research Institute (Olofsdotter et al. 2002) due to its potent application in weed management (Dilday et al. 1998). Screening of allelopathic rice is under investigation and some cultivars in Chinese rice germplasm e.g. Qingkun 2 , Xiayitiao, Jizaoxian showed strong allelopathic effects on barnyardgrass (*Echinochloa crus-galli*), a major weed in paddy fields of Asian countries (Wang et al. 2000; Xu et al. 2003a; Ruan et al. 2005). Rice allelochemicals have been isolated and identified (He et al. 2005a, b, c). The quantitative trait loci (QTL) controlling allelopathic effects in rice have been determined (Zeng et al. 2003; Xu et al. 2002; Xu et al. 2003b). The molecular mechanism of rice allelopathy has been analyzed using differential proteomics (He et al. 2004; He et al. 2005d). The ISSR (inter-simple sequence repeat) molecular marker approach was used by Lin et al. (2005) to estimate genetic diversity in rice and barley allelopathy.

A double haploid (DH) population derived from anther culture of ZYQ8/JX17, a typical indica and japonica hybrid, was used by Zeng et al. (2003) to study genetics of rice allelopathy. The inhibitory effect on the lettuce root growth by water-soluble extraction from leaves of 123 individuals in DH pure lines was investigated, and the QTLs analysis of rice allelopathy was carried out. Four QTLs related to rice allelopathy were detected, and they were on chromosomes 3, 9, 10 and 12, respectively. The research by He et al. (2002b) showed that additive and dominant effects of genes expressed alternatively from 3-leaf stage to 8-leaf stage in rice seedlings. While the additive effect genes played an important role at the 7-leaf stage, the dominant effect genes played the most important role at 3-leaf and 6-leaf stage. However, the two effect genes were equally important at 5-leaf and 8-leaf stage. Rice allelopathy was affected by genotype × environment (GE) interactions (He et al. 2002b).

3.3.3.2 Wheat Allelopathy

There is a tremendous difference of allelopathic effects in various cultivars and genotypes of wheat (Zhang et al. 2006; Zuo et al. 2005a), and even wheat stubbles of different genotypes showed various allelopathic potentials against weeds (Zou et al. 2005b). The allelochemicals in wheat were isolated and identified by Dong et al. (2005). Huang and his Australian collaborators have established good correlations between the phytotoxicity in terms of root growth bioassay upon annual ryegrass (*Lolium rigidum*) and the concentrations of a selection of dynamically produced allelochemicals in the root exudates of cv. Khapli wheat (*Triticum turgidum* sp. *durum* (Desf.) Husn.) (Huang et al. 2003).

3.3.3.3 Allelopathy of Invasive Species

Biological invasion is threatening biodiversity and security of ecosystems. Allelopathy of invasive species is becoming another important research area and attracts many Chinese scientists to work on it (Luo 2005). Allelopathy has been identified as one of the most important mechanisms of invasive success of alien plant species (Callaway and Aschehoug 2000; Weir et al. 2003; Bais et al. 2003). Recent studies have demonstrated that novel biochemical weapons provide invasive alien plant species with an advantage to compete with native species (Callaway and Aschehoug 2000; Weir et al. 2003; Vivanco et al. 2004; Callaway et al. 2004). More than 15 invasive species have been found to have allelopathic potentials in China (Song et al. 2000; Gu et al. 2002; He et al. 2002c; Luo 2005). The in-depth mechanism is under investigation.

3.3.3.4 Microbial Allelopathy

Microbial allelopathy is rarely addressed. Zeng et al. (2001a, b) reported that *Aspergillus japonicus*, a common soil-borne fungus, produces secalonic acid F (SAF), which exhibits strong phytotoxicity and interference with a series of physiological and biochemical processes of receiver plants. The SAF significantly reduced the activities of superoxide dismutase (SOD), peroxidase (POD) and enhanced malondialdehyde (MDA) contents, but lowered the content of chlorophyll a and chlorophyll b, as well as the photosynthetic rate of tested plants (Zeng et al. 2001b). Respiration, membrane permeability and ABA content increased after the treatment of SAF. The SAF treatment also damaged stratiform structure of chloroplast, the membrane and structure of nuclei. *Pisolithus tinctorius* is an ectomycorrhizal fungus that is important for the growth of many tree seedlings. Zeng et al. (2004) also found that the aqueous extracts of *P. tinctorius* fruitbodies inhibited the seedling growth of barnyardgrass, crabgrass (*Digitaria sanguinalis*) and Chinese Pennisetum (*Pennisetum alopecuroides*). Pisolactone isolated from the fruiting bodies of mycorrhizal fungal strongly inhibited the seedling growth of barnyardgrass at a concentration of 100 $\mu g\ ml^{-1}$ (Zeng et al. 2004).

3.3.4 Allelopathy in Modern Taiwan China

Chang-Hung Chou is the pioneer scientist in the field of allelopathy in modern Taiwan China. He systemically investigated autotoxicity of rice straws in a continuous rice monoculture system in the 1970s and 1980s (Chou and Lin 1976; Chou 1986). In southern China, including Taiwan, rice is cultivated twice a year and most rice stubble from the first rice crop is left in the field after harvesting and incorporated into the soil as organic fertilizer. In Taiwan, the yield of second rice crop is about 25% less than the first crop in areas with poor water drainage (Chou 1990). Chou and Lin (1976) reported the phytotoxic effects of decomposing rice straw in the soil on the succeeding rice. Chou and Chiou (1979) found rice straw that was incorporated into soil released high amounts of phytotoxic phenolics and affected the chemical nature of the paddy soil and yield of rice plants in successive crop seasons.

Chou et al. (1981) isolated and identified five phytotoxic phenolics, p-hydroxybenzoic, vanillic, p-coumaric, syringic, and ferulic acids, from a decomposed straw and soil mixture. Chou (1983) found that most of these phenolic compounds from decomposed rice straw were autotoxic to rice.

3.4 Future Directions and Prospect

China has a large number of scientists working on allelopathy. It is estimated that over one hundred research groups have done some work related to allelopathy. Twenty-six Chinese allelopathy scientists participated in the Fourth World Allelopathy Congress in Wagga Wagga, Australia in 2005. Some groups have done in-depth research on allelopathy. The group lead by Xiao-ya Chen published their work in Nature-Biotechnology, one of the top journals in the world in 2004 (Wang et al. 2004). The group lead by Jing-quan Yu in Zhejiang University has done excellent work on plant autotoxicity and published more than ten papers in international journals (Yu and Matsui 1997; Yu 1999; Yu et al. 2000; Yu et al. 2003). The Wenxiong Lin's group has done systemic work on rice allelopathy including germplasm screening, allelochemical isolation and identification, and molecular mechanism. Since 1989 the Shi-ming Luo's group at South China Agricultural University has been working on the allelopathy of invasive alien plants, crops, microbes, plant autotoxicity, allelochemical isolation and identification, and induced and molecular mechanisms of allelopathy (Luo et al. 1995; Luo 2005). The International Symposium on Allelopathy Research and Application was organized by South China Agricultural University in 2004 (Zeng and Luo 2004). Dr. Yong-qing Ma and his group have been working on allelopathy of wheat and weeds since early 1990s (Ma and Han 1993; Ma et al. 1995; Zuo et al. 2005a, b).

Most allelopathy work in China has only showed phytotoxicity of water or solvent extracts of plant materials in the laboratory and could not demonstrate the significance of allelopathy in natural plant communities. The concentrations, release rates, effects of microbial degradation and soil components on the fate of allelochemicals are not clear. Low level repeated study should be avoided. The Ecological significance of allelopathy of investigated species in nature should be justified before an experiment starts (Mallik 2005). Some studies showed phytotoxicity of aqueous leachate and GC/MS analytical results and concluded that the identified compounds were the allelochemicals responsible for phytotoxicity, and the plant has allelopathic effects. Such a conclusion may not be justified since the majority of compounds in water leachates or extracts are not volatile and could not be identified by GC/MS. Methodology for allelopathy research must be appropriate (Inderjit and Callaway 2003). Some well-designed greenhouse experiments should be conducted to distinguish allelopathy from competition. More fieldwork is required to evaluate the actual role of allelopathy in nature (Foy 1999). Allelochemical isolation and identification, and molecular mechanisms should only be carried out for some important crops and then only in those laboratories with good facilities. The actual role of allelopathy in the biological invasion of alien plants should be identified. Indirect effects of allelochemicals on soil chemistry (Inderjit and Weiner 2001), nutrient availability (Inderjit 2001), soil microbial community (Callaway et al. 2004), and symbiotic nitrogen fixation and mycorrhization (Zeng and Mallik 2006) should be investigated.

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Part 2 Allelochemicals and Allelopathic Mechanisms

4. Allelochemicals in Plants

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Abstract. This chapter presents a brief coverage of a range of common plant allelochemical groups, and includes discussion of their structures, chemistry, distribution, ecology, bioactivity, biosynthesis, allelopathy, and mode of action where known.

4.1 Introduction

Allelochemicals are non-nutritional secondary metabolites produced by living organisms (i.e., plants) that have stimulatory or inhibitory effects upon the growth, health, behavior, or population biology of neighboring organisms (plants, insects, microbes, etc.). In plant–plant allelopathy, the more common inhibitor effects are visible upon such plant functions as respiration; photosynthesis; water balance and stomatal function; stem conductance of water; xylem element flux; membrane permeability; cell division and development; protein synthesis; and enzyme activity alteration. Within plants, allelochemicals may be distributed broadly among organs such as seeds, flowers, pollen, leaves, stems, and roots, or sometimes found in just one or two such locations. Their kingdom distribution is rather specific, with a particular class of allelochemical being often found confined to a limited range of plant families or genera, for example, the sesquiterpene lactones found in the Asteraceae. When released from plants, a potential allelopathic compound may first have to undergo some metabolic or environmental alteration in structure before it assumes biological activity. Alternatively, some bioactive compounds released from a plant may be rendered inactive by subsequent chemical transformations in the air, water, or soil. Allelochemicals exist in a vast array of seemingly disconnected structures and possess widely different modes of action. Not many of these modes of action are completely understood, but of those known, it appears that they can be quite divergent from the 30 or so presently known activity modes associated with artificial herbicides. The role played by allelochemicals is mostly interpreted now in an ecological way, usually in some means of plant defense against other plants, pests, or diseases. The standard modes of release for allelochemicals are volatilization, residue decay, leaching, or root exudation. Such exudation has been somewhat overlooked in the past history of allelopathic science, with most attention devoted to the more visible, above-ground aspects of plants. However, the central significance of underground communication and transfer between neighboring plants has received more attention and doubtless will give rise to new endeavors to elucidate allelopathic chemistry and mechanism (Bais et al. 2001). Laboratory bioassays for phytotoxicity/allelopathy are becoming more standardized so that effective comparisons can be made between the allelopathic potentials of different plants (Belz and Hurle 2004). A cautionary comment upon the use of the word "allelochemical" is also warranted. Strictly speaking, this term should really only be applied to those substances whose combat role between donor and receiver plants has been firmly established by quality experimental evidence (especially in genuine field circumstances), and not simply that a plant-derived compound shows toxicity toward some (perhaps irrelevant) other plant during in vitro bioassay. Of course phytotoxicity is a necessary condition for allelopathy, but other criteria must also be fulfilled in order to make the term meaningful. Nevertheless, this chapter has followed the use of the term allelochemical in this broader, less proven way according to much of the published literature. The context of the original information is usually sufficient for the reader to use his/her judgment on the status of the compound.

The deepening of our understanding of allelochemicals in plants holds much promise for widening our knowledge of plant ecosystems. Allelopathy is being increasingly recognized as a contributing explanation for such things as plant dominance, succession, formation of communities, climax vegetation, crop productivity, and exotic plant invasion. Indeed, the quality series of investigations carried out by Bais et al. (2003a) on the exotic invasive effectiveness of spotted knapweed (*Centaurea maculosa*) in the western USA represents the type of integrated study involving ecological, physiological, biochemical, cellular, and genomic sciences that serves as a good example among allelopathy scientists today.

Allelopathy holds out hope for improvements in crop production through such means as discovering eco-friendly herbicides with new sites of action, harmless to crops but toxic to weeds, and without formation of dangerous residues. Finally, the genetic manipulation of crop germplasm to produce crops which carry inwardly all the desirable features of a successful competitor to their weeds, is a strongly motivating goal which is presently being pursued around the world.

4.2 Glucosinolates: Chemistry and Ecology

4.2.1 Structure

The glucosinolates (GSL's) are a chemical class of sulfur-containing, naturally occurring secondary metabolites (β-D-thioglucoside-N-hydroxysulfates) found mainly in the plant order Capparales, and possessing an organic, anionic form usually balanced by cations such as sodium or potassium. Each compound contains a C=N bond capable of E-Z geometrical isomerism and generally accepted as being the Z-isomer on the basis of X-ray crystallographic analysis of one typical representative, sinigrin (Fig. 1). Every member of this class has sulfate attached to the N atom, a thioglucose moiety attached to the C=N carbon, and a varied side chain (R) also attached to the same carbon, thus serving to differentiate individual class members (Brown and Morra 1995; Fahey et al. 2001; Warton et al. 2001).

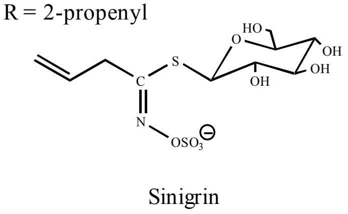


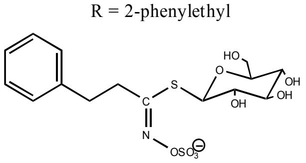
Fig. 1. Sinigrin (2-propenylglucosinolate)

There are at least 120 known GSL's which Fahey et al. (2001) have divided into ten groups on the basis of similarity in structure in the R moiety. These group names and some representative structures are illustrated in Table 1.

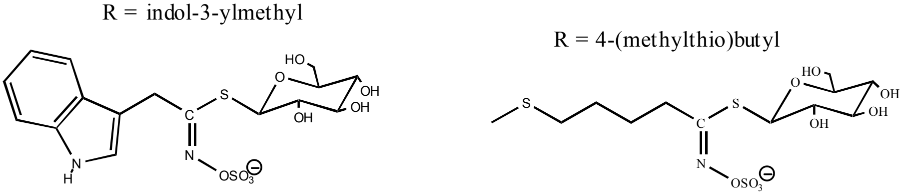
Table 1. The ten glucosinolate structural types

Group Number	Glucosinolate Group Type	Chemical Name of a Group Representative (R)
1	Sulfur-containing side-chains	2-(methylthio)ethyl
2	Aliphatic, straight chain	n-pentyl
3	Aliphatic, branched chain	1-methylethyl
4	Olefins, or alcohols	2(R)-2-hydroxy-3-butenyl
5	Aliphatic straight and branched chain alcohols	1-ethyl-2-hydroxyethyl
6	Aliphatic straight chain ketones	5-oxoheptyl
7	Aromatic	benzyl
8	Hydroxyalkyl benzoates	2-benzoyloxy-1-ethylethyl
9	Indoles	4-hydroxyindol-3-ylmethyl
10	Others	e.g. 3-methoxycarbonylpropyl

Those GSL's containing a sulfur atom in the side chain in various states of oxidation comprise the largest single group (39 compounds), but those with such chiral centers as sulfinyl, like the majority of other chiral carbon centers within glucosinolates, have an (as yet) unknown stereochemistry. The most extensively studied GSL's are a group of 19 from *Brassica* vegetables whose side chains (R) contain such structures as the indole ring, benzene rings, and ω-methylthioalkyl chains, typical examples of which appear in Fig. 2.



Gluconasturtiin



Glucobrassicin

Glucoerucin

Fig. 2. Some common glucosinolates from *Brassica* species

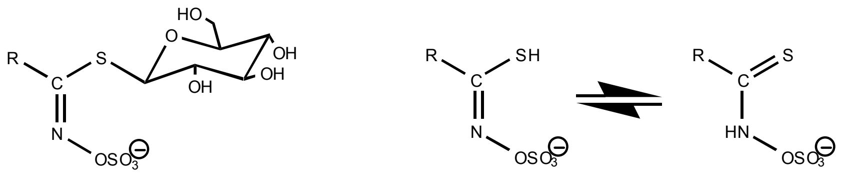
4.2.2 Distribution and Ecology

The first isolation of GSL's goes back to the 1830's when the compounds sinalbin (4-hydroxybenzylglucosinolate) and sinigrin (2-propenylglucosinolate) were obtained from white mustard (*Sinapsis alba*) and black mustard (*Brassica nigra*) respectively. Glucosinolates are now known to be distributed across 16 plant families of dicotyledonous angiosperms (Fahey et al. 2001) with the Brassicaceae containing all ten structural groups and about 96 individual compounds. While the Brassicaceae contains more than 350 genera and 3000 species, GSL's have been found in more than 500 species from other families. The Capparaceae contain eight structure groups and about 19 compounds, while the Resedaceae contain four structure groups and about 11 GSL compounds. With some common overlap, the remaining 13 plant families account for the present total of 120 different GSL structures, some of which have long been known for their allelopathic properties.

The genus *Brassica,* containing numerous edible vegetable crops such as cabbage, choy, kohlrobi, broccoli, swede, turnip, cauliflower, kale, calabrese, Brussels sprouts, and oil crop canola (rapeseed, *Brassica napus*), has been the focus of much chemical attention concerning GSL's (Rosa et al. 1997). Various studies have been conducted on the allelopathic effects of *Brassica* allelochemicals in connection with the growth of lettuce, barnyard grass, and wheat (Oleszek 1987); (Bialy et al. 1990); with growth of velvetleaf (Wolf et al. 1984); and the impact of allyl isothiocyanate released from *Brassica napus* (Choesin and Boerner 1991). The shredded leaves from some *Brassica* species have also been examined for their allelopathic effect upon *Pythium ultimum* and *Rhizoctonia solani* (Charron and Sams 1999). GSL's have been shown to be taken up by a specific carrier system and transported by phloem, so that the internal distribution of GSL's within a plant is usually broad, with most major tissues of stems, leaves, roots, and seeds carrying a proportion of the total. Plant age adversely affects the quantities available, especially after having reached the later vegetative stage. Older plants may contain as little as 1 μmol/g fresh weight, while young sprouts of broccoli (*Brassica oleracea*) can contain up to 100 μmol/g fresh weight. For a given plant organ, variability in GSL type can be quite diverse. For example, with the seeds of radish (*Raphanus sativus*) 4-methylsulfinyl-3-butenylglucosinolate is the main compound, whereas in mustard seed (*Brassica juncea*) allylglucosinolate predominates, while in seeds of cabbage (*Brassica oleracea*) allyl and 2-hydroxy-3-butenylglucosinolate are dominant (Brown and Morra 1997). Environmental factors play a role in determining levels of GSL's in growing plants and may also influence distribution within a plant. Such factors as pest attack, soil fertility, and wounding exert significant effects. Antagonistic interactions between GSL's and bacteria, nematodes, fungi, and herbivores are also well documented in the literature (Fahey et al. 2001). Interestingly, the model genome plant *Arabidopsis thaliana* has been shown to contain 23 different GSL's, which is very high compared to most other plants (Hogge et al. 1988; Haughn et al. 1991). Additionally, as there is a strong interest in controlling the levels of GSL's in crops to improve pest resistance and nutritional value, genes identified in the *A. thaliana* genome program will greatly assist strategies to control GSL's in related *Brassica* crops (Chen and Andreasson 2001). Benefits to allelopathy have already emerged from the growing *A. thaliana* genome information in a study of spotted knapweed (*Centaurea maculosa*) conducted by Bais et al. (2003a) who integrated such data with ecological, physiological, biochemical, and cellular approaches.

4.2.3 Chemistry

Like the cyanogenic glucosides, the initial step in the biosynthesis of GSL's proceeds by hydroxylation of nitrogen in a precursor aminoacid, with subsequent aldoxime formation by decarboxylation. Further steps follow to bring about side-chain modifications and glucone conjugation through the sulfur atom introduced by cysteine. The GSL's are stable water-soluble anions which become easily converted into biologically active degradation products (Fig. 3) such as thiocyanates, isothiocyanates, nitriles, epithionitriles, and oxazolidine-2-thiones, by the enzymatic action of myrosinase (EC 3.2.3.1), a glycoprotein which exists in physical separation from the GSL substrates until such time as the plant cells are broken by wounding, chewing, freeze-thawing, or bruising (Fahey et al. 2001). It is mainly the isothiocyanates (ITC's) which are considered the active allelochemicals arising from the GSL precursors. After the initial hydrolytic cleavage of the sugar moiety by the β–thio-glucosidase, the GSL-aglycone then spontaneously loses the sulfate group to form an unstable intermediate which proceeds to form the abovementioned degradation products. One of the intermediate's major decomposition pathways is via a Lossen rearrangement to form an isothiocyanate, often as the major product (Warton et al. 2001). ITC's interact with proteins irreversibly by attacking their sulfhydryl, disulfide, and amino groups and will therefore destroy enzymes and act as biocides (Brown and Morra 1997).



General glucosinolate structure Intermediate after myrosinase action

General isothiocyanates and oxazolidinethiones General nitriles, thiocyanates, and epithionitriles

Fig. 3. Degradation products from glucosinolates after enzymatic action by myrosinase

4.2.4 Allelopathy

The glucosinolates are considered the precursors of the true allelochemicals possessing the interesting phytotoxicity. Complete GSL anions are water soluble but out of several which have been tested for bioactivity, only 3-indolyl-methylglucosinolate has demonstrated plant growth inhibition (Brown and Morra 1997). Most attention has been given to the ITC's which tend to be the most bioactive and form in the larger amounts during GSL degradation. Thus Tsao et al. (2000) assert that allyl isothiocyanate is considered the actual cause of sinigrin's biological activity. Because a number of the common short side-chain GSL's give rise to low molecular weight isothiocyanates after degradation, these ITC's are relatively volatile and can thus permeate soil pores with some ease. This process has been termed "biofumigation" (Angus et al. 1994; Warton et al. 2001). Activity in the gas phase for allyl isothiocyanate has been demonstrated by germination inhibition of cucumber, dandelion, barnyard grass, soybeans, carrot, and wheat at concentrations near 1 ppm in sealed containers (Brown and Morra 1997). In one study by Brown and Morra (1995, 1996), 22 volatile GSL products made up of ITC's and nitriles were identified in the headspace above *Brassica napus* seed meal, root, leaf and stem tissues, and were shown to be the causative agents for inhibited germination of test seeds in bioassay.

Because glucosinolate-rich plants give rise to active phytotoxins such as ITC's after mechanical damage to cells, green manure mulching (Krishnan et al. 1998), (Petersen et al. 2001) has been one effective way to suppress weed growth without need of artificial herbicides. The volatile ITC's (e.g. butyl-ITC) which possess quite short half-lives in soil, are thought to act as biofumigants on weed seeds within the gas-filled soil pores, while the non-volatile, longer half-life group (e.g. 2-phenylethyl-ITC) which is less susceptible to hydrolysis, continue their herbicidal action within soil solution.

From Petersen's et al. (2001) work using turnip-rape mulch, it appears that all ITC's have the same mode of action and that the primary biological targets are the enzymes of glycolysis and respiration (Drobnica et al. 1977).

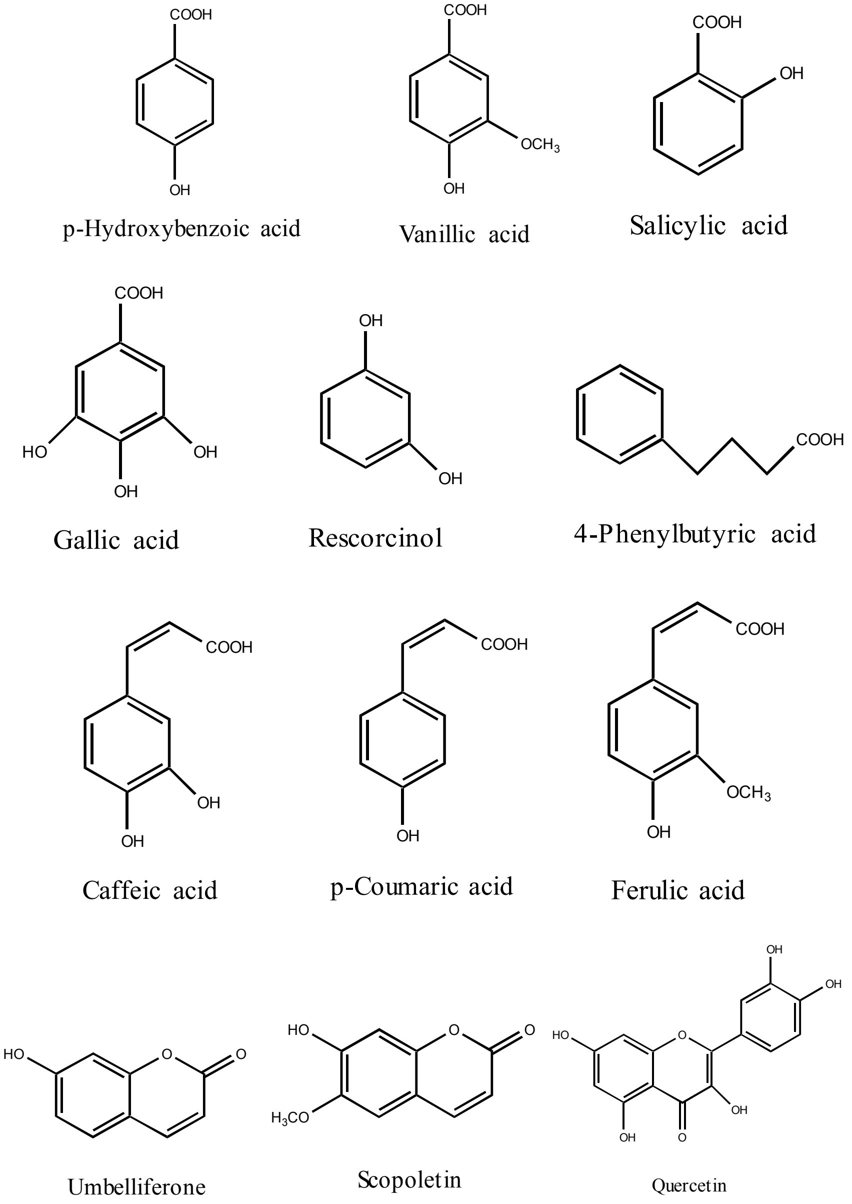
4.3 Phenolic Compounds: Biosynthesis, Distribution, and Chemoecology

4.3.1 Introduction

Within the context of allelopathy, the term "phenolic compounds" has a loose meaning, but is generally thought of as containing a range of compound types that include structures such as simple aromatic phenols, hydroxy and substituted benzoic acids and aldehydes, hydroxy and substituted cinnamic acids, coumarins, tannins, and perhaps a few of the flavonoids. In this chapter, allelopathic flavonoids will be treated separately in the final Section 4.7.2 headed "Other Compounds". An important subset of the phenolics, known as phenolic acids, describes a small range of water-soluble, aromatic, carboxylic group-containing phenols (such as salicylic acid), which are very broadly distributed. Certainly, as a group of allelochemicals, the phenolics have received by far the greatest attention, with a voluminous supporting literature implicating them in some allelopathic activity (Einhellig 2004). Yet in spite of this great body of data, there are properties of this group which do not properly fit the "concept" of an allelochemical. For example, these types of compounds are among the most widely distributed of all plant substances and the least specific in action. Cinnamic acids are considered to be universally present in all higher plants, and benzoic acids have been identified in all angiosperms so far examined (Dalton 1999). They are a common and normal part of most soils. The accumulated evidence points to phenolics as playing a significant role in allelopathy, and a number of reviews on their biochemistry and ecology have been written (Inderjit 1996). However, in the vast majority of cases, these compounds appear as a mixture and not a single substance, and what contribution is made to allelopathy by phenolic compounds is probably never due to a single substance (Einhellig 2004). The different compounds have variable toxicity, have similar modes of action, and attack cellular function at more than one site. Thus they do not display a typical herbicidal response. Their individual concentrations in soil (micromolar) are usually rather lower than what appears by pure chemoassay (0.1–1 mM) to be necessary for significant activity. The weak phytotoxicity they do exhibit seems to be dependent upon additive effects from the individuals in the mixture. Some researchers have sought to invoke the notion of synergism in an attempt to explain the apparent effectiveness of such low-concentration mixtures, but so far no one has been able to duplicate a synergistic mixture of the required activity level. The ordinary natural abundance of phenolics in soils as a standard part of the organic matter is imagined by some, under certain soil conditions, to be able to differentiate the growth of different plants within a neighborhood, yet to date, no experiment has been published which provides conclusive proof of phenolics allelopathy under typical field conditions (Dalton 1999; Huang et al. 2003).

4.3.2 Structure and Distribution

Of the scores of compounds which belong to this phenolics group, a range of typical structures is displayed in Fig. 4.



p-Hydroxybenzoic acid
Vanillic acid
Salicylic acid
Gallic acid
Rescorcinol
4-Phenylbutyric acid
Caffeic acid
p-Coumaric acid
Ferulic acid
Umbelliferone
Scopoletin
Quercetin

Fig. 4. Typical structures among the allelochemical phenolics

Because the phenolics comprise the bulk of the structural matrix of plants and exist in soils through plant decomposition, there exists a continuous ecological cycle of uptake, synthesis, transformation, exudation, and decay involving these substances (Dalton 1999). Soil solutions of free phenolic acids come from plant leachates, root exudates, and bound-form microbial transformations of debris and litter. Loss of free phenolics arises from soil sorption, oxidation, microbial transformation, polymerization, and plant root uptake. Soil microbe action is believed to be the major influence upon the soil solution concentration of phenolics (Blum 2004b). Some workers have perceived simple phenolics as being so readily recycled and metabolized by rhizosphere and soil microorganisms, that naming the substances as allelochemicals can be quite misleading, for their presence can lead to inhibition, to no effect, or even to stimulation of plant growth, depending upon their concentrations and chemical state.

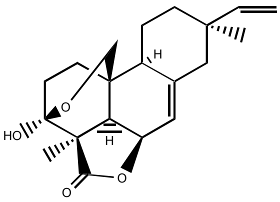
4.3.3 Biosynthesis

Phenolic compounds arise from the shikimic and acetic acid (polyketide) metabolic pathways. The phenylpropanoid group of secondary metabolites (e.g. ferulic acid) recognized by their aromatic ring with a 3-carbon side-chain, is generated via phenylalanine as intermediate, and includes the O-heterocyclic form usually called coumarins (e.g. scopoletin, Fig. 4). Phenylpropanoid compounds play a role in their esterified and glycosidated precursor forms in cell wall lignin production. Certain phenolic acids (e.g. salicylic acid) also play a role in plant physiology, and act as intermediates in acquired disease resistance (Einhellig 2004). The metabolic transformations of phenolics through microbial action are comprehensively discussed by Inderjit et al. (1999). Blum (2004b) concisely describes the bio-conversion by fungi of ferulic acid ultimately into carbon dioxide and water, via such intermediate compounds as vanillic acid, protocatechuic acid, β-carboxymuconic acid, β-oxo-adipic acid, acetic and succinic acids.

4.3.4 Chemoecology and Mode of Action

The actual involvement of phenolics in allelopathic interactions is still controversial despite a vast literature linking them to allelopathic effects (Dalton 1999). There is still no definitive proof of phenolics affecting plant growth under field conditions. Despite the name applied to this group, the *un-substituted* skeleton structures such as simple benzoic and trans-cinnamic acids show greater toxicity than the hydroxylated derivatives (Einhellig et al. 1982). It is not surprising that there appears to be no structure/activity relationship within this group.

Other characteristics of the phenolics are that their inhibitory action is dependent upon concentration (Einhellig 2004); both their primary and secondary effects are readily reversible once they are removed or depleted from the plant rhizosphere (Blum 2004b); and it is soil concentration and not root uptake that appears to be the ultimate determinant of inhibition (Lehman and Blum 1999). They exert multiple physiological effects resulting in a generalized cytotoxicity, often by non-specific permeability changes to cell wall membranes, and do not seem to have a primary target site as do most herbicides (Einhellig 2004). The phenolics may well be working together with much lower concentrations of other more phytotoxic substances passed over unnoticed in the usual complex mixture isolated from plant parts or exudates (Rimando et al. 2001). For example, the composition of rice exudates has been studied for over ten years but despite the presence of phenolic acids in exudates, it is believed that these substances can not explain the experimental observations in allelopathic rice cultivars (Olofsdotter et al. 2002). Later work by Kato-Noguchi (with others) (2002a; 2002b; 2003a; 2003b; 2004) has shown that the trace compound momilactone B (Fig. 5) is probably the true rice allelochemical, perhaps assisted by phenolic action. The natural activity level of momilactone B is as low as 3 micromolar. Additional information on phenolic modes of action in relation to such matters as cell membrane effects, water relationships, phytohormone and enzyme effects, photosynthesis, respiration, and the flow of carbon in plants, can be found in the comprehensive treatment on phenolics by Einhellig (2004).

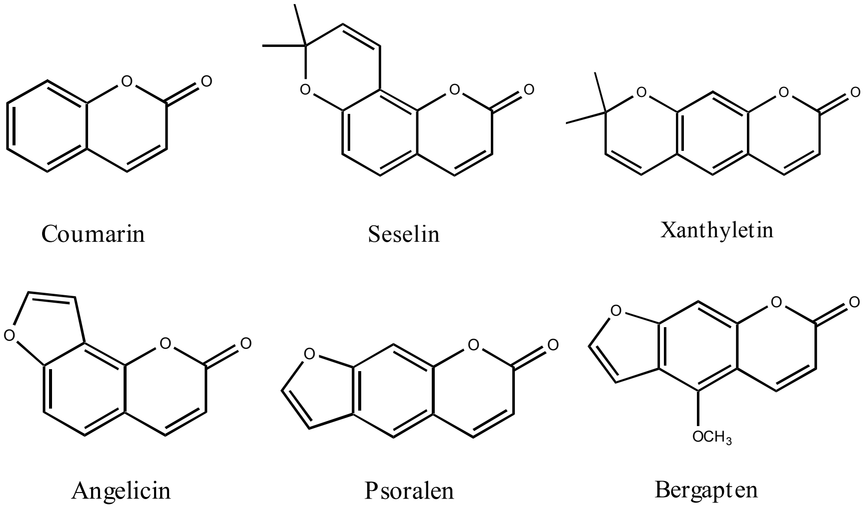


Momilactone B

Fig. 5. The probable diterpenoid allelochemical source of rice allelopathy

The coumarins (as phenylpropanoids) are lactones of a 1,2-benzopyran structure, and are also usually considered a sub-group of the phenolics, with activities very similar to the cinnamic acids, though with some occasional differences. Many coumarins are oxygenated at their 7-position, and have been prenylated by (5-carbon) 3-methylbut-2-enyl units from the mevalonic acid bio-pathway. This mix of biosynthetic paths leads to a vast range of furano- and pyrano- fused-ring coumarins (Fig. 6) possessing an amazing range of bioactivities (Sardari et al. 2000).

Scopoletin, umbelliferone (Fig. 4) and esculetin (6,7-dihydroxycoumarin) make up the most cited group of coumarins linked to allelopathy. As for the apparent additive effect already noted among the phenolic acids, coumarin effects can similarly be enhanced by the presence of a phenolic acid. For example, Korableva et al. (1969) report that scopoletin is more effective as a growth retardant when used in combination with caffeic acid, than when used alone, and Einhellig (1996) reports that a combination of coumarin (umbelliferone), phenolic acid (salicylic acid), and flavonol (rutin) also possesses a stronger effect in combination. While the phenolic acids do not show evidence of being able to influence cell division, compounds such as scopoletin and coumarin have been reported as decreasing mitosis (Avers and Goodwin 1956), and also inhibiting certain enzyme actions (Podbielkowski et al. 1996).



Coumarin Seselin Xanthyletin

Angelicin Psoralen Bergapten

Fig. 6. Typical bioactive coumarins

4.4 Terpenoids: Volatile Allelochemicals

4.4.1 Introduction and Structure

As a chemical class, the terpenoids are distinguished by their origin from the biochemical pathway via mevalonic acid and isopentenyl pyrophosphate (a basic C-5 building unit) which combines with itself to produce C-10 monoterpenes, C-15 sesquiterpenes, C-20 diterpenes, and C-30 triterpenes. Many of the over 24,000 different compounds now known are of a cyclic unsaturated type and carry oxygen at various functional sites, e.g. aldehydes, ketones, ethers, alcohols, and lactones. Stereo-isomers are very common and may possess very different physico-chemical properties from each other. Terpenoid natural functions are very diverse and have been recorded as signal molecules, allelochemicals, phytoalexins, pheromones, visual pigments, photoprotective agents, membrane constituents, and reproductive hormones. A characteristic feature of the C-10 terpenoids is their high volatility and significant odors.

For more than 75 years it has been known that the monoterpenes of essential plant oils have had strong inhibitive effects upon plant growth and seed germination, and that these effects may proceed through the vapor phase. For example, the monoterpene 1,8-cineole (Fig. 7) suppresses the mitochondrial activity of *Avena fatua* coleoptiles and reduces the rate of oxidative phosphorylation (Fischer 1991). The review by Fischer (1986) in a major work of that day published on allelopathy, described mono- and sesqui-terpenes as plant germination and growth regulators, and listed 14 monoterpenes associated with phytotoxicity, including, borneol, pulegone (Fig. 7), camphor (Fig. 7), 1,8-cineole (Fig. 7), limonene (Fig. 7), camphene, and

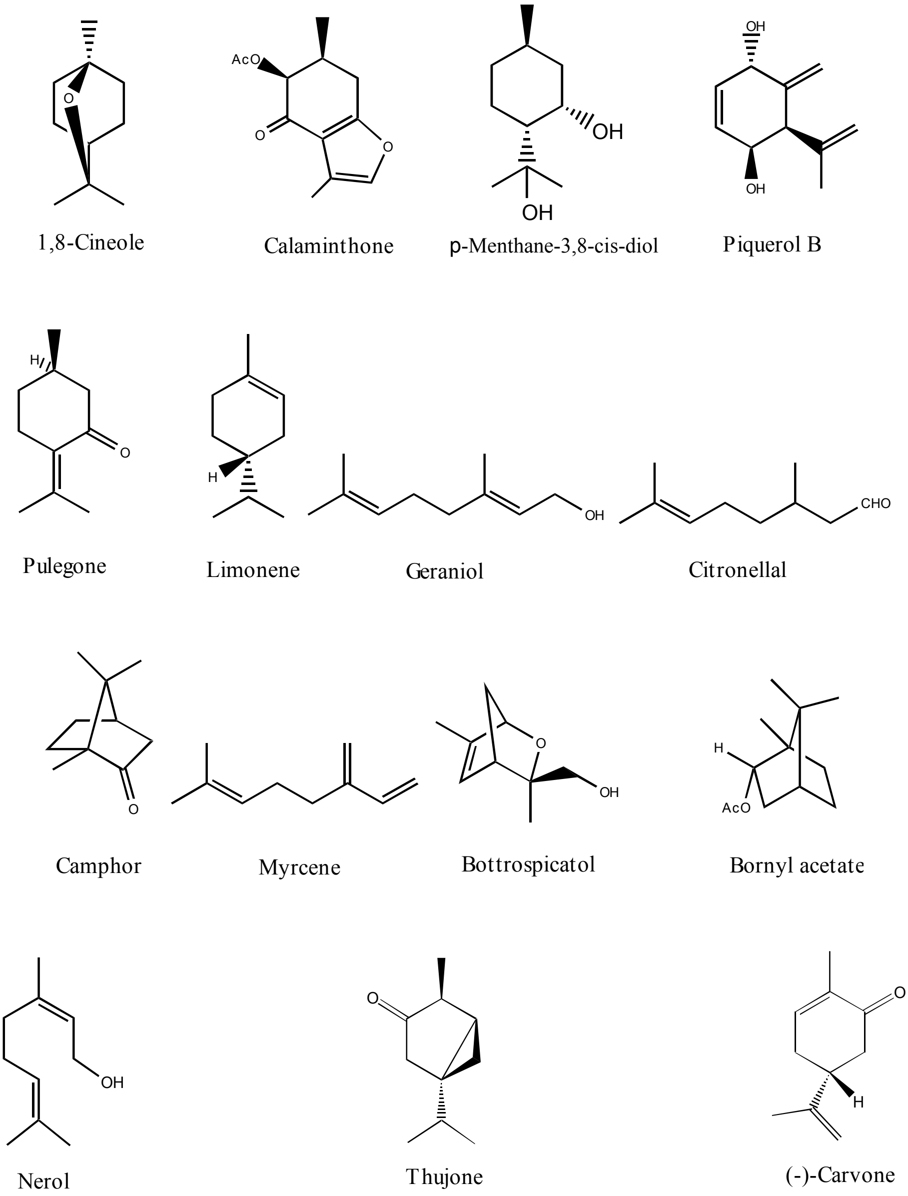


Fig. 7. Typical monoterpenoids

p-cymene. Some workers have at times inferred the phytotoxic action of a particular monoterpenoid within an essential oil, but given that these compounds occur naturally as mixtures, it is now thought that terpenoid toxicity is most probably due to a joint action by two or more compounds combining their activities. Thus in their examination of the effects of the essential oils of rosemary (*Rosmarinus officinalis*), savory (*Satureja montana*), and thyme (*Thymus vulgaris*), against seed germination of radish (*Raphanis sativus*), Angelini et al. (2003) found that the pure principal components of each oil (cineole/borneol; carvacrol; thymol respectively) were not as inhibitive as the essential oil mixture. Some have even suggested a synergistic interaction between oil mixture components, but a recent and comprehensive examination of these ideas by Vokou et al. (2003) with measurements upon germination and growth of *Lactuca sativa* using 47 different monoterpenoids and 11 pairs (in five different proportions) of such compounds, would seem to deny any great significance for the role of synergy in monoterpenoid toxicity. While some degree of synergy did appear for certain pairs, the level of growth inhibition was not comparable to that of a single highly active compound.

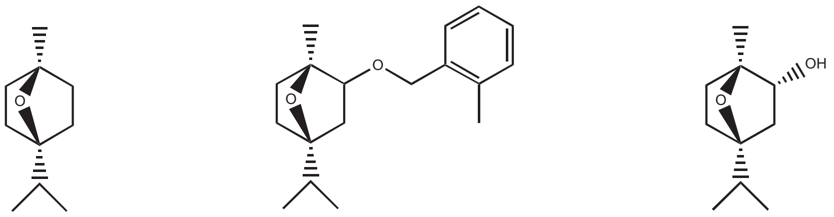
The direct effect of volatile substances from tomato (*Lycopersicon esculentum*) leaves which included such terpenoids as á-terpineol, linalool, thymol, and geraniol (Fig. 7), upon the dry weight of *Lactuca sativa*, was to reduce that weight by 34% (Kim and Kil 2001). GC/MS analysis of the essential oil from tomato plant leaves revealed at least 40 different compounds, of which about 20% were terpenoids. Again, it would be difficult to attempt to assign this toxicity to any one compound.

4.4.2 Monoterpenoid Chemistry and Mode of Action

While it had been long believed that the monoterpenoids were essentially hydrophobic compounds, more recent investigations have actually shown that the terpene hydrocarbons were low (10–20 ppm) in solubility, but that ethers, ketones, and alcohols showed surprisingly high water solubility, e.g., (saturated solutions at room temperature): camphor (11.3 mM) (Fig. 7); borneol (4.2 mM); piperitone (16.8 mM); pulegone (9.2 mM) (Fig. 7); α-terpineol (12.9 mM); menthol (2.7 mM) – concentrations which are higher than those normally used in germination and growth bioassays (Fischer 1991). In fact, Reynolds (1987) has reported comparative phytotoxic effects of aqueous solutions of a series of open chain, cyclic, and bicyclic terpenes for their activity against the indicator species lettuce. Reynolds found that the unsaturated hydrocarbons such as myrcene (Fig. 7), limonene (Fig. 7), and *p*-cymene required concentrations in excess of 2 mM to cause 50% germination reduction. The open chain, the cyclic, and the bicyclic alcohols (e.g., nerol (Fig. 7), terpinen-4-ol, and borneol) were significantly more active, while greatest bioactivity was associated with unsaturated ketones such as thujone (0.022 mM) (Fig. 7) and piperitone (0.075 mM). The impact of stereochemistry was also observed in these studies, whereby the lettuce germination mechanism was affected by (+)-carvone at 0.052 mM, but only by the (–)-carvone isomer (Fig. 7) at the higher 0.38 mM value. Experiments by Mucciarelli et al. (2001) on the essential oil from peppermint (*Mentha* × *piperita* L.) measuring root and mitochondrial respiration of etiolated seedlings of cucumber (*Cucumis sativus* L.), showed that total oil mixture was effective for 50% inhibition at 324 ppm and 593 ppm respectively, and that (+)-pulegone (Fig. 7) was the most toxic compound with 0.08 mM and 0.12 mM concentrations required respectively for 50% inhibition of root and mitochondrial respiration. Also in this work, an interesting positive correlation was found for (+)-pulegone, (–)-menthone, and (–)-menthol between water solubility and respiratory inhibition.

An interesting comparative study by Vokou et al. (2003) which lists the level of bioactivity against indicator species *Lactuca sativa* for seed germination and seedling growth for 47 different monoterpenoids revealed some interesting findings. First, a range of seven different chemical classes were among the chosen terpenoids: hydrocarbons, alcohols, aldehydes, ketones, ethers, acetates, and phenols. For germination inhibition, the alcohol terpinen-4-ol was the most inhibitory compound, while the ketones pulegone (Fig. 7), dihydrocarvone, and carvone (Fig. 7) were next in effectiveness. As overall classes, ketones were most inhibitory, followed by aldehydes, ethers, alcohols, and phenols, with acetates and hydrocarbons least inhibitory. In respect of *L. sativa* seedling growth, 24 compounds from across all chemical classes except acetates demonstrated strong inhibition, with the highest activities shown by citronellol, linalool, isopulegol, citronellal (Fig. 7), myrtenal, carvone (Fig. 7), and dihydrocarvone. Acetates and hydrocarbons were the least active, although one hydrocarbon [(+)-3-carene] did show strong inhibition. Second, non-oxygenated compounds are less active than oxygenated ones (established in the literature already), but if the oxygen is part of an acetate function then the activity is largely lost. Third, straight chain terpenoids are about as active as cyclic ones. Fourth, aromatic terpenoids do not appear to have any greater germination inhibitory power than their more saturated analogs. Finally, there does not appear to be any obvious structure/activity relationship among the monoterpenoids, and the influence of chirality for a given isomer is usually insignificant.

Despite the vast array of known terpenoid structures, very little use has so far been made of them as lead structures for herbicides (Vaughn and Spencer 1993), and very little is so far known of their modes of action. Probably the best understood mode of action is that of 1,4-cineole. Both 1,8-cineole (Fig. 7) and 1,4-cineole (Fig. 8) are strong growth inhibitors, but only the 1,8- isomer inhibits all stages of mitosis. The cineoles are commonly found as components of essential oils from aromatic plants such as *Artemisia* spp. and *Eucalyptus* spp. The 1,4-isomer causes growth abnormalities in shoots, unlike the 1,8-compound (eucalyptol), and so despite the great similarity of structure, the two compounds have different modes of action. The molecular target of 1,4-cineole has been determined to be a critical nitrogen-mobilizing enzyme asparagine synthetase (Romagni et al. 2000). The commercial herbicide cinmethylin (Fig. 8) has a structure based upon that of 1,4-cineole. The benzyl ether group acts to reduce compound volatility and to promote formation (through metabolization of the cinmethylin proherbicide) of the more active *cis*-2-hydroxy-1,4-cineole phytotoxin (Fig. 8) (Duke and Oliva 2004). This hydroxycineole under bioassay against asparagine synthetase activity demonstrated an I_{50} value of 0.03 ìM, more than ten times lower than that of the parent 1,4-cineole.



1,4-Cineole Cinmethylin cis-2-Hydroxy-1,4-cineole

Fig. 8. The bicyclic terpenoid lead structure (1,4-cineole) and its commercial herbicide analogs

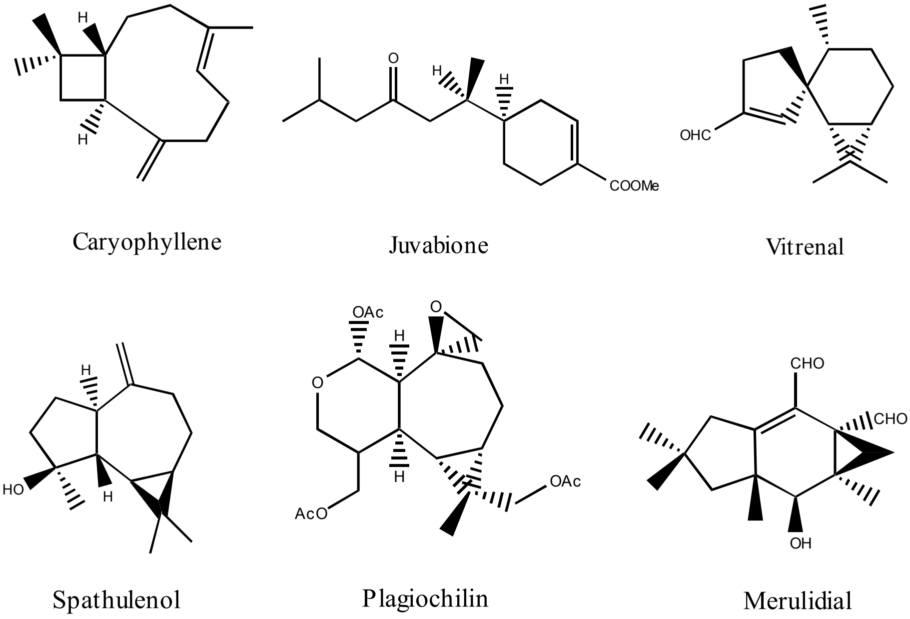
From an allelopathic perspective, the nature of soil transformations upon terpenoid structures holds considerable interest. The fate of both bioactive and inactive terpenes within the rhizosphere soil has received a modest level of investigation, but only little with an allelopathic purpose (Inderjit et al. 1999). However, Nishimura et al. (1983) found that (–)-*cis*-carveol is transformed into (+)-bottrospicatol (Fig. 7) by soil *Streptomyces bottropensis* and that the metabolite was active against lettuce seed germination. This field deserves more investigation in the future.

4.4.3 Sesquiterpenoid Activity

Many C-15 sesquiterpenoids and metabolized derivatives are bioactive across a wide range of organisms and deserve at least a brief survey in relation to phytotoxicity and possible allelopathic effects.

The sesquiterpenoids often accompany the monoterpenes within essential oils, but there are much greater numbers of the sesquiterpenoids. These C-15 compounds are biosynthetically derived from their common farnesyl pyrophosphate intermediate, and because of their higher molecular weights, are found in the less volatile oil fractions. Some typical sesquiterpenoids are depicted in Fig. 9.

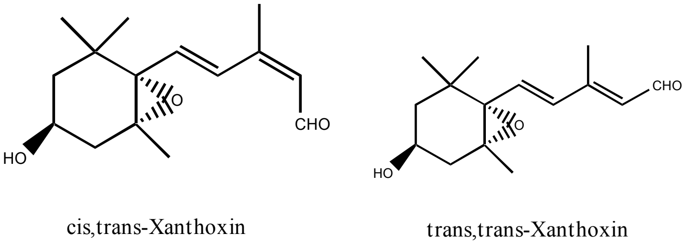
Some of the ecological roles found for sesquiterpenoids include hormonal defense, pollination attraction, herbivory defense, antecedent activity, phytoalexins defense, and pheromonal mimicking (Harborne 1991). While the large sub-group of sesquiterpene lactones (> 5000) seems to have drawn the most attention for their bioactivity, the non-lactone group has demonstrated some limited levels of phytotoxicity. For example, the liverwort *Plagiochila ovalifolia* was found to contain plagiochilin (Fig. 9), which at the 50 ppm level, strongly inhibited the growth of rice seedlings. Structure/activity studies pointed to the acetyl hemiacetal group as being necessary for activity (Matsuo et al. 1981). A more recent example of sesquiterpenes showing putative allelopathic activity is that of the two geometric isomers *cis, trans*- and *trans*, *trans*-xanthoxin (Fig. 10) which were extracted with methanol from the leaves of *Pueraria thunbergiana,* where they were present at concentrations of 51.4 and 72.5 ng/g fresh weights respectively. The xanthoxins inhibited the root growth of cress (*Lepidium sativum*) seedlings at lowest concentrations of 0.3 ìM (*cis, trans*) and 3.0 ìM (*trans, trans*), while the 50% inhibitions of cress roots were measured at 1.1 and 14.0 ìM respectively (Kato-Noguchi 2003c). Very little is presently known about sesquiterpenoid modes of action or molecular target sites.



Caryophyllene Juvabione Vitrenal

Spathulenol Plagiochilin Merulidial

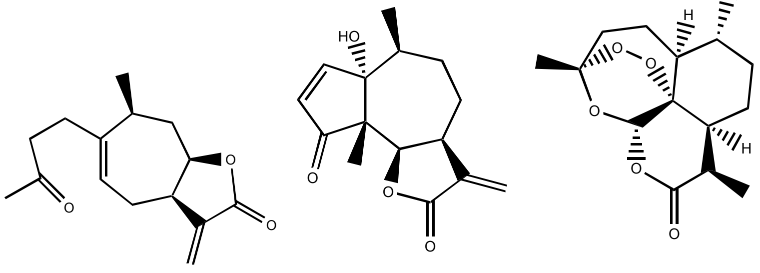
Fig. 9. Typical sesquiterpenoids



cis,trans-Xanthoxin trans,trans-Xanthoxin

Fig. 10. Sesquiterpenoid allelochemicals from *Pueraria thunbergiana*

The much larger group of sesquiterpenoid lactones (SL's) mostly carry an α-methylene-γ-lactone moiety, and are found in over 15 plant families, especially in the Asteraceae (Fig. 11).



Tomentosin Parthenin Artemisinin

Fig. 11. Typical growth regulating sesquiterpenoid lactones (SL)

The SL's also often contain such additional groups as epoxides, esters, unsaturated aldehydes and ketones which have good alkylating properties, and so may easily react with biological nucleophiles such as the thiol groups of plant enzymes. This gives the SL's a broad range of biological activities, including plant growth regulation and phytotoxicity (Fischer 1991).

Some useful examples of phytotoxic SL's include tomentosin, parthenin, and artemisinin (Fig. 11).

Tomentosin is a lactone isolated from the seeds of *Iva axillaris* (poverty weed) by Spencer et al. (1984) which demonstrates a capacity to inhibit both growth and germination of velvet leaf (*Abutilon theophrasti*), a major agricultural weed. Parthenin was studied by Fischer et al. (1989) at levels of 1, 10, and 100 ìM for its action on sorghum, which it was able to significantly inhibit from germination, even at 1 ìM. Such high activity levels are above many other naturally occurring inhibitor compounds. Parthenin's potential allelopathic and herbicidal properties were further explored by Batish et al. (2002) for its effect upon two weedy species *Avena fatua* and *Bidens pilosa*. Parthenin inhibited the root and shoot length and seedling dry weight of both weeds, and continued to do so even when the weeds were grown in soil amended with different concentrations of parthenin. A reduction in chlorophyll content in the growing seedlings was also noticed. The authors recommended further exploration of parthenin as a potential herbicide for future weed management studies. Artemisinin (Fig. 11) is a sesquiterpenoid lactone obtained from annual wormwood (*Artemisia annua*) and possesses an unusual peroxide moiety. Besides being highly active against malarial parasites, artemisinin is strongly phytotoxic, but its mode of action is still unknown despite a series of studies by different groups (Duke and Oliva 2004).

4.5 Alkaloids: Chemistry and Ecology

4.5.1 Introduction

One group of secondary metabolites known from ancient times and given the name "alkaloids" by Meissner, the apothecary from Halle in 1819, because of their alkali-like behavior during extraction from plant sources using aqueous acid, today numbers in excess of 12,000 compounds, of which only some 600 have been examined for their biochemical properties (Wink 1998). These substances are usually cyclic or polycyclic, and are mostly derived through biosynthetic pathways which begin with a natural aminoacid. They therefore always contain at least one nitrogen atom and will often demonstrate the basicity of an amine. Their chemical structures are very diverse and no core fragment can represent the class. Roberts and Wink (1998) state that it is possible to recognize four main groups:

a) alkaloids derived from various aminoacids, e.g. ornithine; lysine; histidine; tryptophan,
b) purine alkaloids, such as the xanthine caffeine,
c) aminated terpenes, such as aconitine (diterpene) and solanine (triterpene), and
d) polyketide alkaloids, e.g. coniine, where nitrogen is introduced into a polyketide skeleton.

While each separate alkaloid can be given a full systematic name, it is much more convenient to refer to them by their trivial names, often related to their original plant source. Some common examples would be the compounds displayed in Fig. 12.

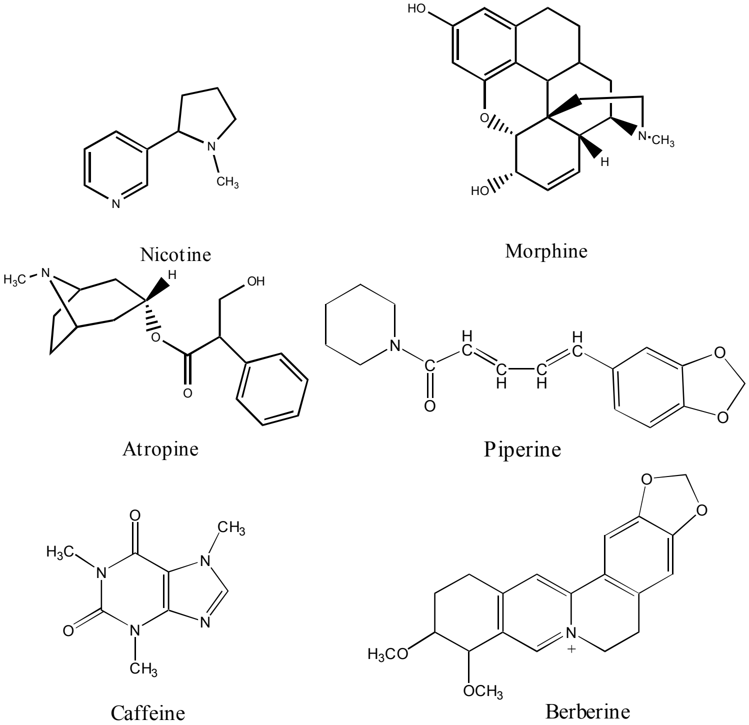


Fig. 12. Typical plant alkaloids

Plant alkaloids are found widely distributed in the families Fabaceae, Apocynaceae, Asteraceae, and Borginaceae, and often fulfill the role of plant defensive agent against fungi, viruses, herbivores, microorganisms, and competeing plants. Some plant species are especially insecticidal because of a range of defensive alkaloids they produce, including such example compounds as strychnine, ephedrine, piperine (Fig. 12), nicotine (Fig. 12), and gramine (Fig. 13). The insecticide nicotine has been used commercially over a long period as protection against commercially important insect pests, while the alkaloid caffeine (Fig. 12) is also quite insecticidal and is probably the most widely distributed compound of this class (Blum 2004a). Such alkaloids as morphine (Fig. 12), berberine (Fig. 12), ergotamine, coniine (Fig. 13), quinine (Fig. 13), and colchicine (Fig. 13) among a group of at least 50 tested compounds possess phytotoxicity, acting to inhibit germination and/or seedling growth in neighboring plants.

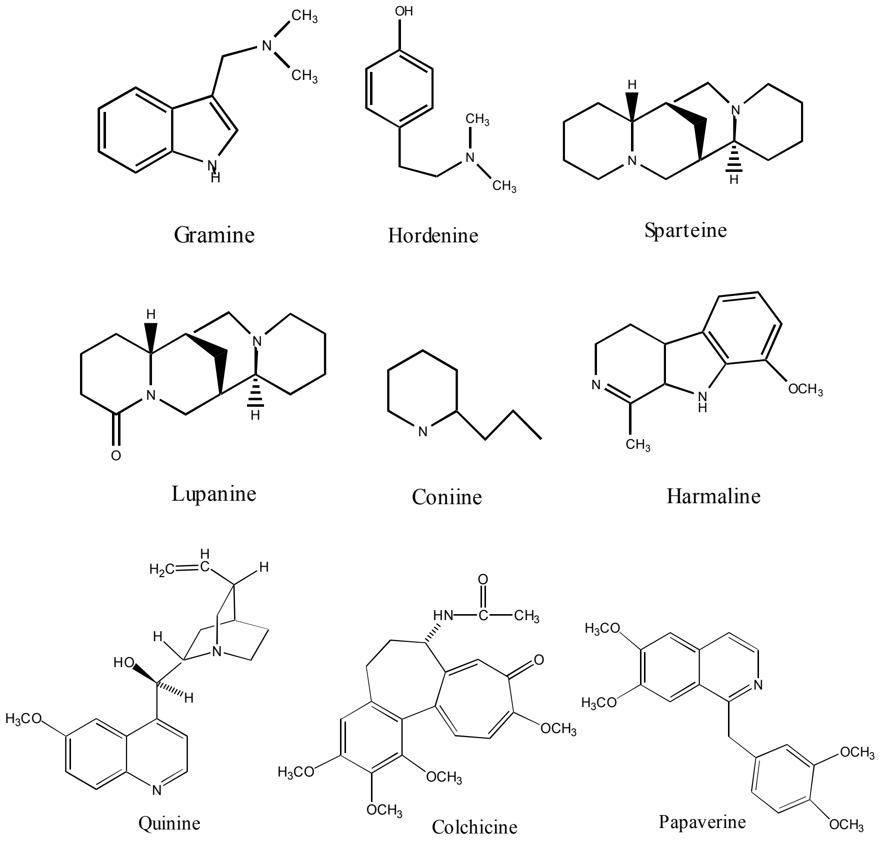

Gramine
Hordenine
Sparteine
Lupanine
Coniine
Harmaline
Quinine
Colchicine
Papaverine

Fig. 13. Some alkaloids which demonstrate phytotoxicity

4.5.2 Modes of Allelopathic Action

While a high proportion of phytotoxic alkaloids attacks more than one plant target site at a time, it is known that the range of targets includes key enzyme functions, photosynthesis, respiration, transcription, protein synthesis, membrane stability, signal transduction, electron transport, and replication (Wink and Latz-Bruning 1995). Thus, a variety of alkaloids binds to or intercalates with DNA or DNA/RNA processing enzymes and can disrupt replication or transcription, e.g. berberine (Fig. 12) and quinine (Fig. 13). Lupanine (Fig. 13) and tubulosine interfere with protein synthesis, while disruption of biomembranes and transport processes is carried out by berbamine and tetrandrine (Blum 2004a). These types of molecular processes are manifested in the outcome from plant bioassays, such as the impact of the four alkaloids colchicine (Fig. 13), harmaline (Fig. 13), papaverine (Fig. 13), and salsoline upon the root growth of *Lepidium sativum*, where 0.01% solutions of these substances reduced radicle length to around 50% or less of the control (Wink and Latz-Bruning 1995). Likewise, in more recent work on the sensitivity of the same test plant *L. sativum* towards the growth inhibiting alkaloids 3''''-oxo-juliprosopine and secojuliprosopinal isolated from the leaves of mesquite (*Prosopis juliflora* (Sw.) DC.), the respective I_{50} values were found to be 0.4 mM and 0.5 mM (Nakano et al. 2004).

4.5.3 Examples of Allelopathic Action

Conclusive experimental evidence linking plant alkaloid production with significant allelopathic field activity remains small.

The alkaloids scopolamine and hyoscyamine were recognized as present in the leachates from seeds of thornapple (*Datura stramonium*) which was studied for its allelopathic potential. Both these compounds were isolated from the soil at a field site overgrown with this plant (Lovett et al. 1981). Levitt and Lovett (1984) showed later that the inhibitory effect of thornapple seeds and leachate on *Helianthus annuus* seedlings remained for a period of eight months in a black earth soil under field conditions.

Two more detailed and specific cases among the alkaloid allelochemicals will be described as examples of plant–plant allelopathy.

The first example consists of the work of the Lovett group at Armidale, Australia, (see e.g. Lovett and Hoult 1995) who, from about 1987–1995 investigated self-defense in the crop barley (*Hordeum* spp), based upon some earlier exploration by Overland (1966). These workers found that living barley plants, hydroponically grown, released the alkaloids gramine (Fig. 13) and hordenine (Fig. 13) from their root systems. The alkaloids were identified and quantified by HPLC (Hoult and Lovett 1993), and their phytotoxicity against white mustard (*Sinapsis alba*) confirmed in chemoassay (Lovett et al. 1989) and biological assay. By four days after germination, barley exudate of hordenine had reached 48 ppm, a concentration which gave significant reduction in radicle length on test plant *S. alba*. The gramine in barley exudate maximized at 22 ppm and also contributed to radicle reduction in *S. alba*. Release of these allelochemicals was observed over a growth period of 75 days (Liu and Lovett 1993a). In a control hydroponics solution in which barley had not been grown, no affect on the bioassay test species was observed. Radicle length response by white mustard to the different doses of each alkaloid followed the classic curve depicting hormesis at very low concentration (a stimulation above control) followed by growing levels of inhibition as the concentration of allelochemical increased. Physical examination by electron microscopy of the root tips of *S. alba* after subjection to the alkaloids, evidenced the same type of cellular disruption as is commonly observed in such cells under other types of stress (Liu and Lovett 1993b). Significantly, these workers have also demonstrated that at 250 ppm, gramine is also able to severely inhibit the growth of white mustard in soil. A genetic investigation (Lovett et al. 1994) into hordenine production by 43 various barley lines (ancestral, landraces, middle eastern, and modern) under two types of environmental conditions, found that environmental conditions during growth had more influence over alkaloid production than did genetic factors. Highest hordenine levels were measured at 327 ìg/g dry weight barley root.

The second example relates to the quinolizidine alkaloids, prominent among legumes. The quinolizidine alkaloids (QA) biosynthesis from aminoacid lysine via cadaverine to representatives sparteine (Fig. 13) and lupanine (Fig. 13) occurs within the green above-ground plant parts, and is thus regulated by light (Wink 2004). The seeds of such plants can be especially rich in alkaloids and may reach levels of 8% by dry weight. Within lupins, QA's have been measured at flowers 4%; fruit 3.9%; pollen 4.7%; leaf 4%; epidermis 6% dry weight; phloem 5 mg/mL; and xylem 0.05 mg/mL. The various bioactivities of QA's consist of such properties as inhibiting the multiplication of potato-X virus and the growth of bacteria and fungi; having repellant, toxic, or mutagenic actions toward nematodes, cows, snails, aphids, bees, beetles, and locusts; and phytotoxicity towards germination and growth of certain plants (Wink 1983 and 1985). The role of these alkaloids appears to be one of general plant defense. The effects of QA's observed against plants are probably due to their interference with membrane integrity and with protein synthesis.

4.6 Hydroxamic Acids: From Among the Benzoxazinoids

4.6.1 Structure

The allelochemicals often called "cyclic hydroxamic acids" are actually a small part of the broader group of naturally occurring benzoxazinones which possess the 2-hydroxy-2H-1,4-benzoxazin-3-(4H)-one skeleton as shown in Fig. 14 (Sicker and Schulz 2002).

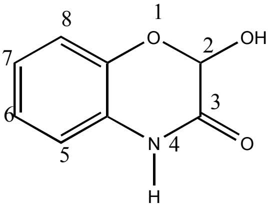


Fig. 14. The 2-hydroxy-2H-1,4-benzoxazin-3-(4H)-one skeleton

Whilst certain variable levels of bioactivity have been found among a range of the broader benzoxazinone group, it is those particular members which possess the special labile combination of cyclic hemiacetal unit (at position 2) and a hydroxamic acid component (the N of the heterocyclic ring at position 4 carries an hydroxyl group) together, which are recognized as the key bioactive compounds for allelopathy (Fig. 15).

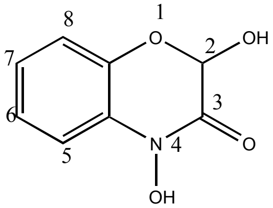
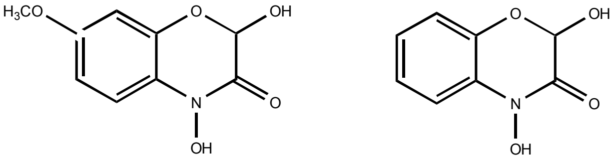


Fig. 15. The labile, bioactive, cyclic hydroxamic acid skeleton

Two prominent allelochemicals of this hydroxamic acid class are the compounds known mostly by their acronyms as DIMBOA and DIBOA (Fig. 16).

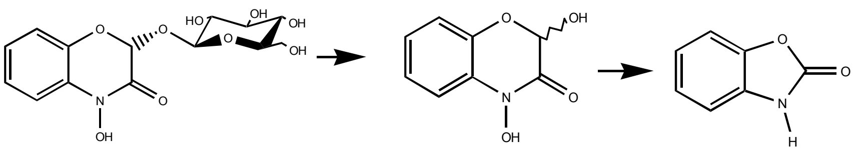


(a) DIMBOA (b) DIBOA

(a) DIMBOA [2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-(4H)-one],
(b) DIBOA [2,4-dihydroxy-2H-1,4-benzoxazin-3-(4H)-one]

Fig. 16. Prominent hydroxamic acid allelochemicals

The benzoxazinones (6-membered heterocyclic ring) are a sub-group of the wider class of natural products with the name benzoxazinoids whose members also embrace the structural group called benzoxazolinones (5-membered heterocyclic ring), some of which arise from the natural degradation of benzoxazinones. The first such benzoxazolinone (BOA) to be recognized in nature was from rye, where an elevated level of resistance towards pathogenic fungi was noticed. Soon after, BOA was realized to be the degradation product of DIBOA, which in its turn was formed from its stable, non-active 2-β-D-glucoside by enzymatic hydrolysis (Virtanen and Hietala 1960) (Fig. 17).

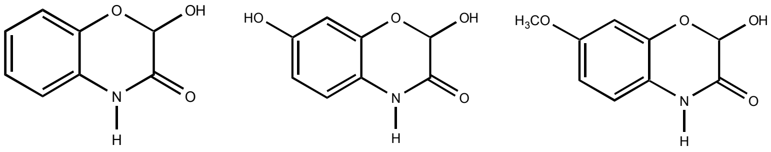


(a) DIBOA-Glc (b) DIBOA (c) BOA

(a) (2R)-2-β-D-glucopyranosyloxy-4-hydroxy-2H-1,4-benzoxazin-3(4H)-one [DIBOA-Glc], (b) 2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one [DIBOA], (c) Benzoxazolin-2(3H)-one [BOA]

Fig. 17. Biosynthetic pathway in rye to BOA

All the natural reported glycosides of the benzoxazinoids have a D-glucose moiety [Glc-] at the 2-position with a (2R)-2-β-linkage. The only other known hydroxamic acids are DIM_2BOA (2,4-dihydroxy-7,8-dimethoxy-2H-1,4-benzoxazin-3(4H)-one) and TRIBOA (2,4,7-trihydroxy-2H-1,4-benzoxazin-3(4H)-one) from *Zea mays* (Sicker and Schulz 2002). One natural derivative of a hydroxamic acid (HDMBOA) 2-hydroxy-4,7-dimethoxy-2H-1,4-benzoxazin-3(4H)-one from maize and wheat still shows high bioactivity and lability like the free hydroxamic acids (Friebe 2001). All the benzoxazinone glucosides are biologically inactive. However, if they possess the hydroxamic acid-like structure after hydrolysis through disruption of the plant cell, they become bioactive in the aglucone form (Mizutani 1999). The range of natural benzoxazinones which have a simple hydrogen atom attached to the N atom at position 4 (the lactams) such as HBOA, DHBOA, or HMBOA, do not display significant bioactivity (Fig. 18).



HBOA DHBOA HMBOA

Fig. 18. Natural benzoxazinones of the lactam type

4.6.2 Distribution and Ecology

Benzoxazinone compounds (usually conjugated with glucose) occur naturally within the plant families Acanthaceae, Poaceae, Ranunculaceae, and Scrophulariaceae (Sicker and Schulz 2002), and hold a special place of interest because such bioactive compounds appear to act as defense chemicals for their host against a range of attacking organisms such as insects, microbes, and other plants (Niemeyer 1988; Wilkes et al. 1999). This property has stimulated wide interest in enhancing such ability within important crops such as maize, wheat, and rye which carry these compounds. The stable, inactive benzoxazinone glycoside can undergo a two-step degradation, first through a glucosidase hydrolysis to the active heterocyclic aglycone – a process enabled by plant cell injury, then through a simple chemical ring contraction to the benzoxazolinone, during which time chemical attack occurs on key enzymes of the attacking organism. For example, in maize, the enzymatic release of the active DIMBOA in response to attack by insects occurs within 30 minutes from injury. The content of DIMBOA in maize shoots has been measured at 1–10 mmol/kg fresh weight (Sicker and Schulz 2002), so that moderately high quantities are available for defense. Known occurrences for the glucosides of common benzoxazinones include the species HM_2BOA in *Zea mays*, HMBOA in *Secale cereale*, DHBOA in *Coix lachryma jobi*, HBOA in *Blepharis edulis*, HDMBOA in *Triticum aestivum*, DIM_2BOA in *Zea mays*, DIMBOA in *Triticum aestivum*, and DIBOA in *Consolida orientalis*.

The hydroxamic acids have been found in plant roots as well as aerial parts, and also in the exudates from selected cereals but appear to be absent from the seed (Niemeyer and Perez 1995). Compound levels usually increase quickly after germination, reaching a maximum in just a few days and then more slowly decrease with increasing plant age (Copaja et al. 1999; Burgos et al. 1999; Cambier et al. 2000). Absolute levels will also depend upon the cultivar and environmental growth conditions (Niemeyer 1988; Wu et al. 2001a; Friebe 2001).

4.6.3 Chemistry and Biosynthesis

Biosynthesis of plant benzoxazinoid compounds depends on the tryptophan pathway from anthranilic acid to indole, where the specialized production of the secondary pathway is capable of forming indolin-2-one. Further monooxygenase hydroxylations to 3-hydroxyindolin-2-one and then to 4-deoxy-DIBOA, form the lactam precursor of DIBOA at the end of this biosynthetic process (Mizutani 1999; Desai et al. 1996). The methoxyl group of related DIMBOA stems from various C-1 sources such as methionine, glycine, and glycerate (Niemeyer 1988).

Because of the hemi-acetal function at position 2 on the benzoxazolone ring, there is a natural equilibration between this form and the open-ring hydroxy-aldehyde structure (Fig. 19). Such tautomerism leads to incapacity to isolate either pure (2R)- or (2S)- forms. For DIMBOA (Fig. 16), the spontaneous ring contraction to MBOA (6-methoxybenzoxazolin-2-(3H)-one) occurs with overall loss of formic acid, probably through an intermediate isocyanate (Niemeyer 1988), and with a half-life in broken maize cells of about one day. The presence of a 7-MeO- group in DIMBOA enhances the reactivity of its heterocyclic ring above that for the unsubstituted DIBOA. Heating of an aqueous solution of DIMBOA is sufficient to convert it to MBOA. In China, roots from the plant *Coix lachryma jobi* L. (var. ma-yuen Stapf) known to contain the anti-inflammation agents MBOA and DIBOA have been applied as therapy for neuralgia and rheumatism (Sicker and Schulz 2002).

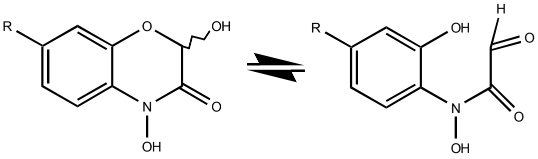


Fig. 19. Tautomeric equilibrium in the 2-hydroxybenzoxazolones

4.6.4 Structure/Activity and Mechanism of Molecular Action

In contrast to other common classes of acetal glycosides known to the chemical literature, the feature of the natural benzoxazinones is the presence of a nitrogen atom within the hemiacetal ring, which imparts an amount of instability to the aglucone, leading to the characteristic bio-activity. The evidence to date favours the need for both a 2-hydroxy and a 4-hydroxy group to be present on the benzoxazinone ring (Hashimoto and Shudo 1996), in which case, according to one model, a multi-centered cationic electrophile can form after metabolic O-acylation and heterolytic cleavage of the N-O bond (Sicker and Schulz 2002). The Sicker group (2002) have themselves proposed a possible alternative mechanism based upon the creation of a 3-formylbenzoxazolinone formed by dehydration of the cyclic hydroxamic acid aglucone. Such an electrophilic formyl group donor would be a strong interactor with typical nucleophilic centers in the enzymes of other organisms (Friebe 2001).

4.6.5 Allelopathy

Benzoxazinoid-containing plants have been known for quite some time to have significant allelopathic potential, and important crop types like rye have been closely investigated for their phytotoxic effects on other plants, especially weeds, as this effect might possibly be tailored to become one ingredient of a weed management system (Friebe 2001; Barnes and Putnam 1987). This allelopathic effect of rye on the weeds crabgrass and barnyard grass was attributed to the DIBOA isolated, together with its less bioactive degradation product BOA. Because the benzoxazinoids can be released from plant residues, the strategy of mulching has been often used as a weed control. Some crop cultivars of different species can exude benzoxazinoids from their roots directly into soil from the living plant. Considerable attention has been directed towards the development of crops (e.g. wheat) which may only require low applications of herbicide to control weeds (Wu et al. 2000; Wu et al. 2001b; Wu et al. 2002; Huang et al. 2003). Another class of less active allelochemicals is often found mixed with hydroxamic acids in plant root exudates, viz., the phenolic acids. Growing evidence suggests that these two classes of compounds may work together to generate an allelopathic effect greater than either class on its own (Huang et al. 2003). Hydroxamic acids have also been found in weeds such as quackgrass and barnyard grass (Pheto 1993). Quackgrass is an aggressive perennial and its allelopathic effect has been described using the growth of cress in a root exudate recirculating system (Schulz et al. 1994) where the phytotoxicity was at least partly attributed to the DIBOA content. Strongly evidential molecular-level explanations for allelopathic effects are rare in the literature, but a small body of useful data is beginning to be built. One such notable example is the study by Friebe et al. (1997) who showed that 0.25 mM solutions of DIBOA or DIMBOA inhibited the crucial enzyme H^+-ATPase which acts as the electrogenic pump of the plasma membrane maintaining a proton gradient required for the transport of ions and molecules, and thus is tied closely to a plant's root growth. Many allelopathic bioassays are based upon root growth inhibition (or stimulation) and therefore offer an indirect measure of allelochemical action upon key enzymes such as that above. The Friebe et al.

(1997) study performed dose-response measurements using both DIBOA and BOA over the range 0–5 mM and measured both radicle elongation and ATPase activity. They found remarkable parallelism between the two separate response measurements, even to the extent of observing the same typical allelochemical stimulation of oat root (*Avena sativa*) growth at low dose concentrations coinciding with corresponding stimulated ATPase activity.

Six naturally occurring benzoxazolin-2(3H)-ones have been recorded in a total of seven plant species, although all of these compounds have been reported as present in *Zea mays* (Sicker et al. 2004). Besides BOA (Fig. 17) and MBOA, there are also DMBOA (6,7-dimethoxybenzoxazolin-2(3H)-one), 4-ABOA (4-acetylbenzoxazolin-2(3H)-one), 5-Cl-MBOA (5-chloro-6-methoxybenzoxazolin-2(3H)-one), and 4-Cl-DMBOA (4-chloro-6,7-dimethoxybenzoxazolin-2(3H)-one)). While the first three have been shown to have a glucosidic origin, the biosynthetic source for the latter three has not yet been established. Some compounds like BOA and MBOA have demonstrated bioactivity against pests, and show phytotoxicity against a range of weeds and crops (Sicker et al. 2004). However, it appears that for compounds such as BOA, its phytotoxic ability can be overcome in some plants (such as oats) because they possess ability to detoxify it (Perez 1990). BOA absorbed from aqueous solution into oat roots, was found to be enzymatically converted to 6-hydroxy-BOA and thence to inactive BOA-6-O-glucoside. Direct glucosylation of the ring nitrogen was also an additional pathway for detoxification within oats (Wieland et al. 1998). One final and interesting fact about BOA (and MBOA) is its relatively easy conversion by soil bacteria, such as *Acinetobacter calcoaceticus*, *Waksmania aerata*, and *Pseudomonas iodina* into simple 2-aminophenol, which in air undergoes a dimeric oxidation to the dark red compound 2-amino-3H-phenoxazin-3-one (Fig. 20), also known independently as the antibiotic questiomycin A from *Streptomyces thioluteus*. This aminophenoxazinone demonstrated higher phytotoxicity than its precursor BOA against such weeds as barnyard grass, and therefore the allelopathic effect of plant mulch rich in BOA (such as rye) must be seen to be enhanced by this extension of soil metabolic processes (Gagliardo and Chilton 1992).

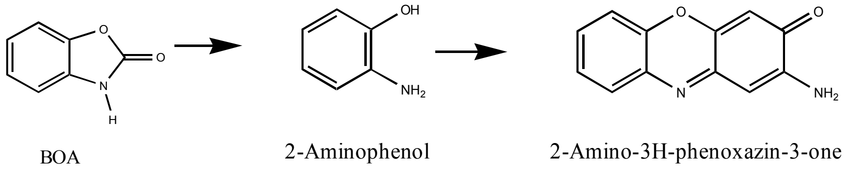


Fig. 20. Bacterial and aerial transformation of the benzoxazinoid BOA to questiomycin **A**

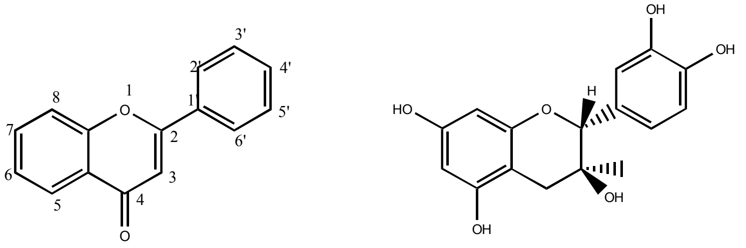
4.7 Other Compounds

4.7.1 Introduction

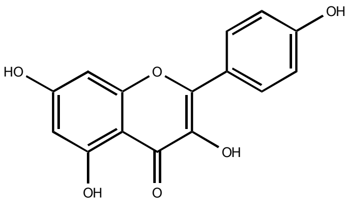
The above classes of allelochemicals by no means cover all that is presently known about plant allelochemicals. New discoveries of fresh plant–plant systems are being constantly made and published, revealing an ever-widening list of different structural types demonstrating phytotoxicity, and where it can be reasonably proved, mediating a true allelopathic effect. In this section, an attempt is made to introduce some of the common smaller groups of compounds associated with allelopathy.

4.7.2 Flavonoids

The flavonoids are a large group of compounds having a central (flavone) structure as depicted by typical examples in Fig. 21. Flavonoids are widely distributed in the plant kingdom, especially among the woody species.



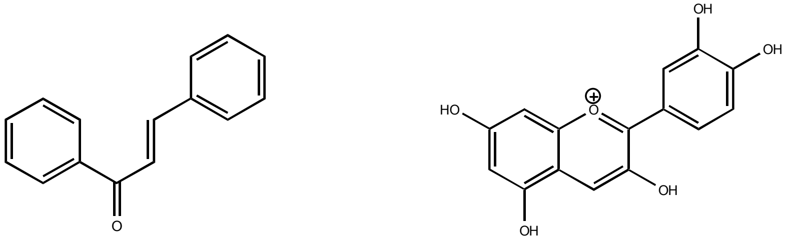
Flavone (+)-Catechin



Kaempferol

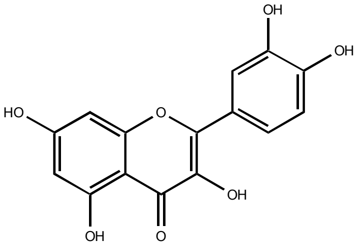
Fig. 21. Typical flavonoids

Only a small group has been shown so far to be implicated in allelopathy. The ring structure of flavonoids is such as to readily adopt the formation of a stable free radical, which can easily lead to the formation of dimers and oligomers and condensed tannins, from among which, a few phytotoxic compounds are known. The biosynthetic origin of the flavonoids arises from a shikimic acid metabolite (such as *p*-coumaric acid) acting as a starter acid for a triplicate acylation with malonyl-CoA to form a chalcone intermediate (Fig. 22), which then undergoes a simple cyclizing 1,4-conjugate addition by phenolic oxygen on one aromatic ring to the enone system, thereby creating the 6-ring oxygen heterocycle of a flavonoid. This central oxygen heterocycle is easily oxidized to the aromatic heterocycle characterizing the flavones (yellow to orange colors), while further dehydration of this ring system leads to the oxonium ion heterocycle of the anthocyanidins (reds and blues), e.g. cyanidin (Fig. 22). These flavonoid derivatives constitute many of the vivid colors seen in flowers, fruits, and autumn leaves.



Chalcone

Cyanidin



Quercetin

Fig. 22. Compounds related to the flavonoids

Such flavonoids as quercetin (Fig. 22) and its 3-O-rhamnoside (quercitrin), isolated from the leaves of the noxious perennial weed *Pluchea lanceolata* have been shown to inhibit the growth of the legume asparagus bean (*Vigna unguiculata*) at concentrations of 10^{-4} to 10^{-3} molar (Inderjit and Dakshini 1995). The allelopathic potential of *Cistus ladanifer* has also been examined because its root exudate was found to contain the flavone apigenin-4′-(O)-methyl and the flavonol kaempferol-3,7-di(O)-methyl which at concentrations of 0.5 and 1.0 mM respectively, could inhibit seedling growth of the herb *Rumex crispus* (Chaves et al. 2001).

The flavan-3-ols are one of the most prominent of the phytotoxic flavonoids, and among these, catechin (3,5,7,3′,4′-pentahydroxy-2-phenylbenzopyran) has received much attention (Fig. 21). In this structure, the 2-phenyl substituent and the 3-hydroxy substituent are *trans* to each other. The free radical scavenging and antioxidant ability of the flavan-3-ols has been recorded (Feucht and Treutter 1999) and their role recognized as first-aid molecules limiting cellular damage from free radicals formed in stress situations. The redox action of catechin itself is stated by Erdei et al. (1994) as being the reason for its inhibition of membrane-bound H^+-ATPase. In a review of the small amount of allelopathy literature on flavonoids, Einhellig (2004) states that the flavonoids are the second most active class of allelochemicals inhibiting mitochondrial oxygen uptake, and that only the quinone class was more effective in this role. Work by Moreland and Novitsky (1987) led them to conclude that flavonoids act primarily as electron transport inhibitors through perturbation of the mitochondrial inner membrane. In a series of recent papers by Bais and co-workers (e.g. Bais et al. 2003a,b; Weir et al. 2003), the unusually high level of toxicity of (–)-catechin was explored against other flavonoids with a range of weeds. Thus Bais et al. (2003b) assayed the flavonoids (–)-catechin, naringenin, kaempferol (Fig. 21), quercetin (Fig. 22), (–)-epicatechin, (+)-epicatechin, and (±)-dihydroquercetin against germination, root and shoot length in the weeds *Centaurea maculosa*, (spotted knapweed), *Centaurea diffusa* (diffuse knapweed), *Linaria dalmatica* (dalmatian toad flax), and *Kochia scoparia* (kochia), together with crop plants wheat and tomato. They found that the minimum inhibitory concentrations for bioassay for all flavonoids were about 250 lg/mL, except for (–)-catechin, which had a value five times less. This confirmed further their earlier findings that the phytotoxic root exudate from invasive *C. maculosa* contained (±)-catechin, and that the (–)-isomer was the one with potency. Some interesting structure/activity observations were also made in this work, in that the phytotoxic (–)-catechin and (+)-epicatechin both have a 2S configuration, and that the 2,3-*trans* relationship between substituents in (–)-catechin appears to provide additional potency. In the Weir et al. (2003) catechin studies, the relationship between concentration of (–)-catechin within root exudate of spotted knapweed (*Centaurea maculosa*) and the capacity of that weed to displace native plant communities was examined. These researchers measured a 5-week growth curve to determine the variation of (–)-catechin concentration in knapweed exudates as the plant matured and discovered that catechin concentration rapidly grew to a maximum in the first two weeks then slowly tapered off to less than 50% of that maximum after a further three weeks. While some test plants were significantly inhibited in growth with as little as 50 lg/mL of catechin, the donor weed itself (*C. maculosa*) did not show any significant effects until catechin reached 600 ìg/mL or more, thereby indicating that under natural field conditions, secretion by the weed does not occur at levels which are autotoxic. The follow-up studies by Bais et al. (2003a) which examined the mechanism by which (–)-catechin helped spotted knapweed to be such a successfully invasive species in North America, subsequently revealed that this allelochemical triggers a wave of reactive oxygen species (ROS) initiated at the root meristem, which leads to a Ca^{2+} signaling cascade triggering genome-wide changes in gene expression, finally leading to death of the root system. When 200 ìg/g soil (dry weight) of (–)-catechin was added to natural field soil in pots, the germination of two native North American grasses (*Festuca idahoensis* and *Koeleria micrantha*) was severely reduced. This series of studies on the invasive effectiveness of spotted knapweed in western USA is one of the best published studies on allelopathy in the literature, and admirably integrates data from ecological and other sources.

4.7.3 Quinones

While natural quinones are widespread in the plant kingdom, very few have been studied for their implication in allelopathy. Nevertheless, numerous references to the allelopathic phenomenon in the literature will have cited a classic situation involving the quinone known as juglone (Fig. 23), an exuded, amber-colored compound from the black walnut tree (*Juglans nigra*) reported by Massey (1925). Massey observed that walnut caused wilting and dying of alfalfa, tomato, and potato. Davis (1928) associated the observed phytotoxicity from walnut with the compound 5-hydroxy-1,4-naphthoquinone (juglone) and reported its toxicity against alfalfa and tomato. Some of the more significant plant quinones that have been associated with allelopathy are shown in Fig. 23.

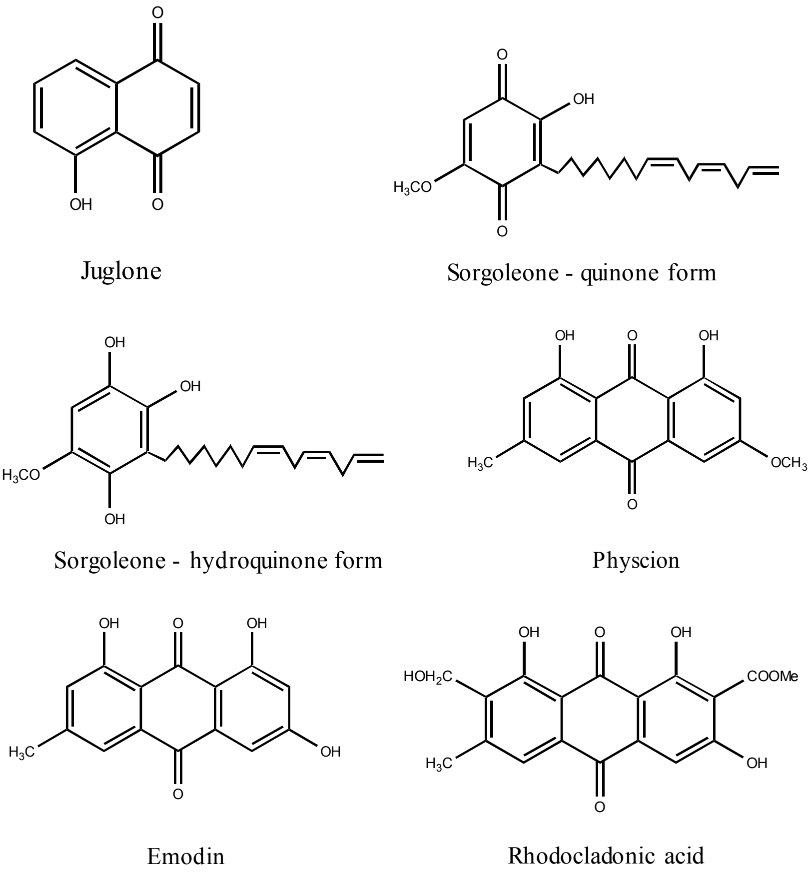


Fig. 23. Examples of phytotoxic quinones

Among these quinones, juglone has the longest history of study (Massey 1925); (Lee and Campbell 1969); (Rietveld 1983); (Duroux et al. 1998); (Hejl and Koster 2004). It occurs as the colorless, non-toxic, reduced form (hydrojuglone) inside living tissue. Contact with atmospheric oxygen immediately oxidizes hydrojuglone to its toxic quinonoid form (Rietveld 1983). High phytotoxicity at micromolar levels has been reported for juglone against a large number of plant species (Hejl and Koster 2004). Juglone concentration levels for activity have been variously described as 10 ppm for inhibition of growth in *Alnus glutinosa*, and of germination in

Rudbeckia hirta, and *Lactuca sativa*; 1.3 ìM for inhibition of key enzyme *p*-hydroxyphenylpyruvate dioxygenase; and 2 ìM for increased oxygen uptake in isolated soybean and corn mitochondria (Hejl et al. 1993). Several earlier studies on the mechanism by which juglone exerts its toxicity, strongly suggested that it disrupts the electron transport functions in mitochondria and chloroplasts, but while Hejl and Koster (2004) agree that the symptoms of such processes are apparent in juglone-affected plants, they do not see this activity as the prime mechanistic action, but as a secondary outcome induced by juglone's action on H^+-ATPase in root cells, which induces loss of water uptake and hence stomatal closure with reduction of photosynthesis.

Another quinone which has received heavy attention for its allelopathic nature is sorgoleone (Fig. 23). This compound was first reported as an isolate from the root exudate of *Sorghum bicolor* (Netzley and Butler 1986) but is also available from other related sorghum species. It is quite hydrophobic and can be easily extracted from roots by methylene chloride. Across a number of sorghum cultivars, a remarkable 76–99% of root exudate consisted of sorgoleone-type compounds (Weston and Czarnota 2001); (Kagan et al. 2003), with some cultivars yielding as much as 15 mg/g fresh root weight. Studies across various laboratories point strongly to sorgoleone as being a potent inhibitor of photosynthesis with site of inhibition within the PSII complex. Sorgoleone has been shown to be quite phytotoxic towards velvetleaf, crabgrass, and barnyardgrass. A concentration-dependent growth reduction using sorgoleone in soil was observed against *Amaranthus retroflexus*, *Lactuca sativa*, and *Portulaca oleracea*, while fresh weight and shoot length of *Abutilon theophrasti*, *Cassia obtusifolia*, and *Portulaca oleracea* were reduced at sorgoleone concentrations of 40 ppm or less. However, crabgrass (*Digitaria sanguinalis*) and green foxtail (*Setaria viridis*) were little affected at such dilute concentrations (Weston and Czarnota 2001). Strictly speaking, the actual exudate from *Sorghum bicolor* does not consist just of the quinone sorgoleone with a C-15 side-chain, but also small amounts of other similar quinones with C-17 or C-5 side-chains (saturated and unsaturated) and with small differences on the ring moiety, such as ethoxyl replacing methoxyl, or acetyloxy replacing hydroxyl groups. However, the PSII inhibiting abilities of these minor quinones were very similar to that of the main structure, suggesting that all these sorgoleone congeners contribute to the overall allelopathy of the sorghum plant (Kagan et al. 2003). Like juglone, sorgoleone also has a reduced hydro-form (Fig. 23) (Fate et al. 1990) which is exuded from sorghum roots along with the main quinone, but is very easily oxidized to the active quinonoid structure. In recent times Dayan et al. (2003) have unraveled the biosynthetic pathway for sorgoleone biosynthesis using C^{13} NMR techniques. Both the C-15 side-chain and the quinone head of this molecule are derived from acetate units, but each moiety is biosynthesized in a different subcellular compartment. A 5-pentadecatriene resorcinol intermediate has been recognized as being the product of a polyketide synthase, and this alkylresorcinol methylated by an O-methyltransferase (using S-adenosylmethionine) then dihydroxylated by a P450 monooxygenase to produce the final hydrosorgoleone structure.

Other quinones demonstrating activity are the anthraquinones such as physcion, emodin, and rhodocladonic acid (Fig. 23). The latter two compounds have been isolated from lichen sources and appear to have an adverse effect upon the PSII system of plants. The first two compounds have been implicated in the allelochemical interactions of *Polygonum sachalinense* (Inoue et al. 1992), while bioactivities of emodin and physcion on target species *Lactuca sativa, Amaranthus viridus, and Phleum pratense* have been measured at 10–100 ppm (Macias 1995). Four-month-old defoliated leaves of *P. sachalinense* were found to contain large amounts of emodin (213 mg/kg) and physcion (180 mg/kg dry wt.), and the soil levels of these two quinones at the center of a *P. sachalinense* community were measured at 55 and 30 mg/kg dry wt. respectively, large enough to inhibit growth of all three test species.

4.7.4 Polyacetylenes

The biological activity of this small group of compounds mainly from the Compositae family has been reviewed by Towers and Wat (1978) who found them toxic to organisms of various types. Russian knapweed (*Centaurea repens*) has been shown by Stevens (1986) to contain at least five polyacetylenes, the structure of which at least one (Fig. 24) was shown to be very phytotoxic.

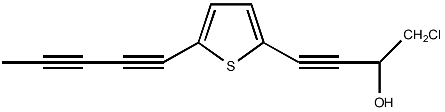
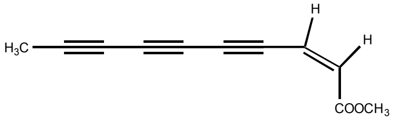


Fig. 24. A thiophene-containing phytotoxic polyacetylene found in the roots of *Centaurea repens*

A few other phytotoxic polyacetylenes are known, such as those from the roots of the Asteraceae, of which the plant *Solidago altissima* is one. From this species, a C-10 compound, *cis*-dehydromatricaria ester (*cis*-DME) has been isolated (Kobayashi et al. 1980; Lam et al. 1992; Inoguchi et al. 2003) and shown to possess strong growth inhibitory effects on other plants (Kobayashi et al. 1980; Tsao and Eto 1996). While *cis*-DME (Fig. 25) is considered an allelochemical of *S. altissima,* its biosynthesis and mode of action are still unknown.



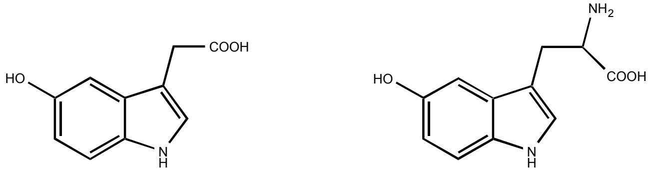
cis-Dehydromatricaria ester

Fig. 25. The allelopathic polyacetylene of *Solidago altissima* – *cis*-DME

4.7.5 Miscellaneous

New allelopathic compound discoveries from plant sources over the past 20 years of allelopathic science have proceeded at an escalating pace, particularly since general access to powerful mixture-separation and spectroscopic analysis instruments, such as GC/MS/MS; LC/MS/MS; HRMS; FTIR; HPLC-PDA; ^{1}H and ^{13}C-NMR has become available. These techniques have enabled plant scientists and natural products chemists to work with the often very small amounts of substance available in the complex mixtures obtained from plant and soil sources in order to determine the chemical structure of bioactive components present. Four representative studies along these lines across the years 1989 to 2003 are summarized below.

Hagin (1989), working on a base of earlier enquiries into the source of allelopathic activity belonging to the highly competitive perennial grass-weed quackgrass (*Agropyron repens* L. Beauv.), carried out quackgrass extractions and used bioassay-guided separations to eventually isolate and identify two key compounds responsible for inhibiting growth in other plants. These allelochemicals were identified as 5-hydroxyindole-3-acetic acid (5-HIAA) and 5-hydroxytryptophan (5-HTP) (Fig. 26), using TLC, HPLC, MS, UV, and IR, analysis techniques. The effects of these two auxin-like compounds were examined against test plants corn and kidney bean over the range 10^{-5} to 10^{-11} M. 5-HIAA stimulated corn root growth at levels below 10^{-6} M but had no effect on shoot growth. 5-HTP stimulated corn root growth with a maximum at 10^{-8} M and shoot growth with a maximum at 10^{-9} M. 5-HIAA had no effect on kidney bean shoot growth in the range 10^{-11} to 10^{-5} M, but became inhibitory at values above 10^{-4} M. It inhibited kidney bean root growth at all the tested levels.



5-Hydroxyindole-3-acetic acid 5-Hydroxytryptophan

Fig. 26. Allelopathic (stimulatory/inhibitory) compounds from quackgrass

Work published by Yu and Matsui (1993) described the isolation of a novel allelochemical from the root exudate of cucumber (*Cucumis sativus* L.) grown hydroponically, by using a recycled exudate trapping system. The phytotoxic compound they discovered was the simple molecule *para*-thiocyanatophenol (Fig. 27), whose structure was recognized by GC/MS and ^{1}H-NMR after solvent extraction from the XAD-4 trapping resin. This compound isolated from the cucumber exudate solution was established at 0.7 mg/g exudate from the growth over 80 days of three seedlings. Thiocyanatophenol markedly reduced the uptake of nutrient ions by cucumber seedlings, and exhibited an ED_{50} for lettuce roots of approximately 0.1 mM.

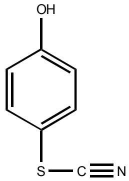
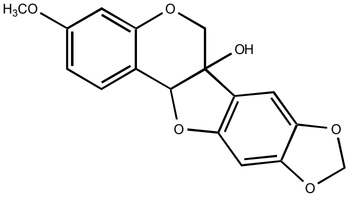


Fig. 27. The thiocyanate allelochemical found in cucumber exudates

The chemical structure of a phytotoxic compound extracted with methanol from pea (*Pisum sativum* L.) shoots and which may play an important allelopathic role in pea residues, was determined recently by the efforts of Kato-Noguchi (2003d), who used MS, IR, and ^{1}H-NMR techniques. The compound, pisatin, has the structure shown in Fig. 28, and demonstrated its toxicity in chemoassay by reducing the growth of cress (*Lepidium sativum* L.) with an ED_{50} as low as 10 ìM.



Pisatin

Fig. 28. Pisatin, a phytotoxin from *Pisum sativum* (pea)

A rather surprising outcome from studies carried out by Kamo et al. (2003) on acetone extracts from the leaves and stems of the legume hairy vetch (*Vicia villosa* Roth) was the discovery of the commercially manufactured nitrogen fertilizer and herbicide cyanamide (H_2NCN) as a phytotoxic natural product. That the cyanamide was indeed a proper metabolite within this legume was confirmed by the fact that 9-day-old seedlings grown without nutrients contained 40 times as much cyanamide as non-germinated seeds. Lettuce seedling hypocotyl length was used as the bioassay to direct the chromatographic fractionation of vetch extract, and while ^{1}H-NMR, ^{13}C-NMR, and IR spectrometries were used for identification of the allelochemical, the simplicity of this molecule gave relatively little spectroscopic data, so that comparison with authentic cyanamide was of considerable help. Quantitative estimation of the cyanamide content in the hairy vetch plants gave a value of 130 lg/g fresh weight.

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5. Allelopathy: Full Circle from Phytotoxicity to Mechanisms of Resistance

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Abstract. Recently there have been important strides in decoding the role of allelopathy in ecological invasions. However, in order to truly unlock the potential of allelopathy it is necessary to understand how allelochemicals affect plants on many levels, from their influence on community dynamics to their cellular targets within an individual plant to how modifications of the structure of a chemical can alter its activity. Collaborations between ecologists, chemists, and molecular biologists are now facilitating the elucidation of some of these interactions. Perhaps one of the most recent and well-documented of these interdisciplinary studies of an allelochemical is the research that has been done on (±)-catechin, a phytotoxin produced by *Centaurea maculosa*. As detailed in this chapter, several ecological, chemical and molecular studies have been conducted in an attempt to clarify the role of this chemical in the invasive success of *C. maculosa*. Although proof for an ecological role of this phytochemical remains elusive, these studies provide a model for the type of interdisciplinary work that is required to determine the importance of allelopathy in ecology and to manipulate the allelopathic potential of certain plants for weed management in cultivated crops.

5.1 Introduction

Higher plants produce a compositionally diverse selection of >100,000 different low-molecular-mass natural products, or secondary metabolites (Dixon 2001); many of which are secreted from roots as root exudates (Walker et al. 2003; Bais et al. 2004). Although roots are traditionally considered for their roles in structural support of the plant and water/nutrient uptake, one of the more remarkable metabolic features of roots is the ability to deposit a vast array of compounds into the rhizosphere. Up to 21% of all photosynthetically fixed carbon is transferred to the rhizosphere through root exudation (Marschner 1995). These root exudates are thought to strongly influence the physical, biochemical, and ecological properties of the rhizosphere. Root exudates can serve as chemical mediators in plant–plant and plant–microbe interactions, in addition to assisting the plant in obtaining sparingly soluble nutrients such as phosphorus (Bais et al. 2004). There is also evidence that roots respond to certain biotic challenges by secreting secondary metabolites, proteins, and volatiles (Bais et al. 2002; Walker et al. 2003; Steeghs et al. 2004). In fact, it is likely that the rich diversity of secondary metabolites arose partly in response to selective pressures for improved defense mechanisms against attacking microbes, insects, and plants. Allelopathy results when a compound produced by one plant species has toxic affects on a neighboring plant species. The naturally produced plant toxins that have been characterized to date are found in many different chemical classes, and display varying physiological effects on susceptible species ranging from suppression of seed germination to inhibition of seedling growth, and damage to meristematic tissues. This suggests that the cellular targets and modes of action of these toxins are also diverse.

Allelochemicals have the potential to be employed for weed management in agro-ecosystems. Like synthetic herbicides, they often exhibit selectivity, and could prove to be an environmentally benign method of weed control when allelopathic plants such as rye, wheat, or sorghum are included in a rotational system or employed as a cover crop (Weston 1996). However, while many plants display allelopathic tendencies, the practical application of allelopathy has been limited. The primary reason for this is a lack of understanding of the mechanisms behind allelopathic selectivity, physiological modes of action, and the genetic regulation of biosynthesis. A better understanding of these characteristics is important to selectively enhance the allelopathic potential of certain crops through traditional breeding methods and genetic engineering (Weston 1996). Enhancing these traits in crop plants may also increase their resistance to insects and soil microorganisms (Walker et al. 2004).

Understanding allelopathic mechanisms is important for the effective utilization of allelochemicals. Until recently, the primary mode of action had not been established for any allelopathic compounds (Einhellig 1995); however, recent and ongoing research is unraveling how some of these chemicals work at the cellular level (Weir et al. 2004). In the past, the diversity of allelochemicals and a lack of multidisciplinary skills among allelopathy researchers have resulted in few research teams with the expertise to lead a sustained effort to identify molecular mechanisms of allelochemicals. However, an increase in interdisciplinary collaborations and emerging technologies such as examining gene expression in model plant systems could lead to a better understanding of the processes mediating plant–plant recognition and communication. The availability of gene chips for plants such as *Arabidopsis thaliana*, *Medicago truncatula*, and *Oryza sativa* and other emerging technologies may facilitate the process of understanding the genetic mechanism associated with these metabolic interactions (Buckhout and Thimm 2003).

In this chapter we focus on research using *C. maculosa* Lam., an allelopathic invasive weed, as a model system to determine the intricacies of plant–plant interactions. To date, research on *C. maculosa* and closely related species has provided us with one of the most complete allelopathy models for an invasive weed. Published works have demonstrated that *C. maculosa* secretes a chemical from its roots that is widely phytotoxic, that this chemical is elicited in response to stresses, and that it is present in the soil (Bais et al. 2002, Bais et al. 2003a). Furthermore, it has been shown that the chemical incites cell signaling responses and genome-wide activation of stress-related genes in susceptible plants such as *A. thaliana* (Bais et al. 2003a). In addition, the allelochemical shows selectivity, as varying plant species that have been challenged with the toxin display a range of susceptibility (Weir et al. 2003; Perry et al. 2005a). However, the arguable downfall of many allelopathy studies is the lack of correlation between laboratory data and actual ecological relevance. We hope that future studies combined with ecological observations may help elucidate the role that allelochemistry plays in invasive weed ecology.

5.2 *Centaurea* Species as a Model System for Studying the Involvement of Allelopathy in Plant Invasions

The primary focus of allelopathic studies has often been crop plants such as *O. sativa* (rice), *Triticum aestivum* (wheat), and *Sorghum bicolor* (sorghum) because the identification of allelopathic compounds from these plants could lead to new crop varieties that can suppress the growth of encroaching weeds and minimize the need for costly applications of commercial herbicides (Weston 1996). However, allelopathy has also been hypothesized as being an important factor in the invasive success of some exotic species. Recent studies of *Centaurea* spp., particularly *C. maculosa* or spotted knapweed, a noxious weed in the western portion of North America, have shed light on both the ecological role and mode of action of the allelochemicals from these plants.

Centaurea maculosa Lam., a member of the Asteraceae family, was presumably introduced into North America as a contaminant in alfalfa and clover seed. Since its introduction, this Eurasian native has become the largest rangeland weed problem in western Montana (Whitson et al. 1999) and is causing growing concern in other parts of the western United States and Canada. Like many invasive species, *C. maculosa* is not a dominant competitor in its natural system, but often establishes monocultures in its new habitat by competitively eradicating native neighbors (Callaway 2002). This leads to a reduction of resource values including, forage production, wildlife habitat, species diversity, and ecosystem function (Jacobs and Sheley 1999). The predominant theory for the success of invasive plants is that they have escaped the natural enemies found in their native ecosystem, freeing them to compete more successfully for limited resources (Keane and Crawley 2002). However, allelopathy has been proposed as an alternate theory for the success of some invasive plants (Callaway and Aschehoug 2000).

Ecologically speaking, it was thought that plant communities were primarily comprised of species having similar adaptations to their physical environment rather than consisting of coevolved species that interact with one another through forms of chemical communication (Callaway 1997). Callaway and Aschehoug (2000) challenged this view by proposing the "novel weapons hypothesis", the concept that the invasive success of some exotic species is due to their ability to bring new mechanisms of interaction to plant communities. They compared the effects of *Centaurea diffusa*, a close relative of *C. maculosa*, on grass species that coexist with the plant in its native habitat in Eurasia and on closely related grass species from North America. They suggested that a component of the root exudates from *C. diffusa* was detrimental to the growth of North American grasses because addition of activated carbon to the soil negated this effect. Furthermore, they observed that the addition of activated carbon reduced the competitive ability of Eurasian grasses against *C. diffusa*, suggesting that the advantage of these Eurasian species is also partially mediated by root-secreted chemicals. Phytotoxic chemicals have recently been identified in the root exudates of *C. diffusa* (Vivanco et al. 2004), and in root exudates of the closely related knapweeds *C. maculosa* (Bais et al. 2002), and *Acroptilon repens* (formerly *Centaurea repens*) (Stermitz et al. 2003), strengthening the hypothesis put forth by Callaway and Aschehoug (2000) that chemical-based interactions may play a role in invasion ecology.

5.2.1 Identification of (±)-Catechin as a Root-Secreted Phytotoxin from *C. maculosa*

While evidence suggested that *Centaurea* invasions had an allelopathic mechanism mediated by root exudation (Callaway and Aschehoug 2000; Ridenour and Callaway 2001) no candidate phytotoxic metabolite had been successfully isolated or characterized in root exudates. To address this issue Bais et al. (2002) used High Pressure Liquid Chromatography (HPLC) separation of sterile-collected root exudates from in vitro grown *C. maculosa* plants coupled with bioassays against several weeds, including *Linaria dalmatica*, *Verbascum thapsus*, *Bromus tectorum*, *Kochia scoparia*, *C. diffusa*, the model plant *A. thaliana*, and crop plants like wheat (*T. aestivum*) and tomato (*Lycopersicon esculentum*) to identify phytotoxic components of the *C. maculosa* exudates. The most effective phytotoxin secreted from the roots of *C. maculosa* was identified as a racemic mixture of (±)-catechin (Bais et al. 2002) (Fig. 1A). Evaluation of (+)- and (–)-catechin separately reveals that (–)-catechin is primarily responsible for phytotoxic effects, while (+)-catechin is a weaker phytotoxin, but a strong antimicrobial (Bais et al. 2002; Veluri et al. 2004). (+)-Catechin, a widespread plant flavonoid is reportedly responsible for many of the beneficial properties of green tea due to antioxidant free radical scavenging (Kim et al. 1997), an antitumour agent (Du et al. 2001), and an insect repellent (Kiderlen et al. 2001). In nature, (–)-catechin is found much less frequently than the (+) isomer (Nahrstedt et al. 1987) or the racemic form (Karimdzhanov et al. 1997). The bioactive properties of both of these compounds infer their ecological significance, with each enantiomer potentially contributing to *C. maculosa's* invasive success. The stress-inducible secretion of racemic catechin from *C. maculosa* roots (Bais et al. 2002) reinforces the hypothesis that these chemicals are important for both aggression and defense.

In the laboratory, (±)-catechin has shown broad-spectrum activity against a variety of plant species, including closely related *C. diffusa* and the model plant *A. thaliana* (Fig. 2A); however, at concentrations determined to be ecologically significant, the toxin had no effect on *C. maculosa* (Bais et al. 2002; Weir et al. 2003; Perry et al. 2005a). Racemic catechin has also been identified in soil extracts from *C. maculosa* invaded fields, linking invasions to exudation of (±)-catechin in the soil (Bais et al. 2002; Bais et al. 2003a; Perry et al. 2005b). However, inherent soil properties, varying soil collection, storage and extraction methodologies, and seasonal variation in secretion patterns has made actual quantification of soil catechin difficult and unreliable, resulting in huge variation in reported values and resulting in uncertainty of the ecological importance of this phytotoxin in plant invasions (Perry et al. 2005b; Blair et al. 2006; Perry et al. 2007).

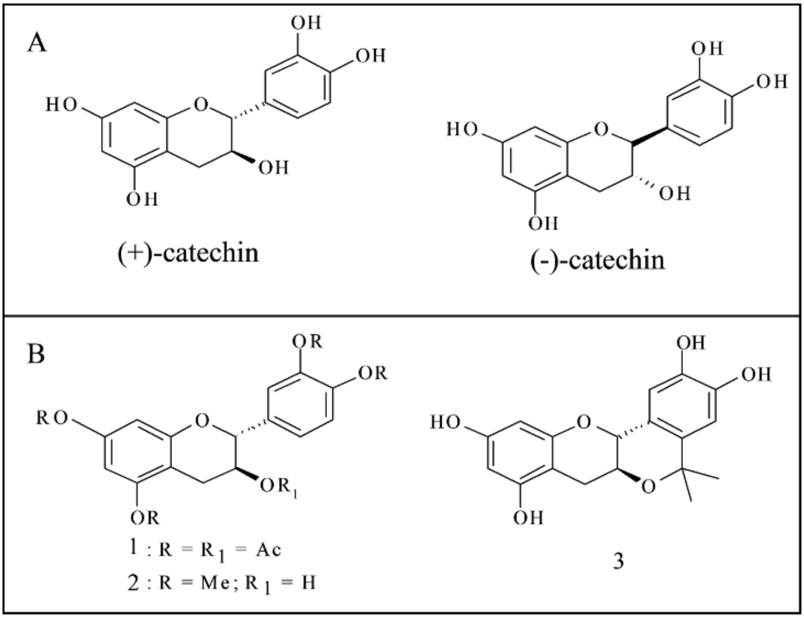

Fig. 1. Chemical structure of (+) and (–)-catechin, and the synthetic pentaacetylated (1), methoxylated (2), and cyclized (3) derivatives of (+)-catechin

5.2.2 Structure-Dependent Phytotoxicity of (±)-Catechin

Published studies have provided clues suggesting that the structure of (–)-catechin is important for its phytotoxic properties. The potent phytotoxicity observed for (–)-catechin is relatively rare for a natural flavonoid. Several compounds and intermediates in the flavonoid pathway, such as naringenin, kaempferol, quercetin, (–)-epicatechin, (+)-epicatechin, and (±)-dihydroquercetin have been assayed for their effect on shoot and root differentiation and germination efficiency in various plants, and were found to be much less phytotoxic than (–)-catechin (Bais et al. 2003b). Interestingly, (+)-epicatechin was the only flavonoid tested that showed activity against *C. maculosa*. Both enantiomers of epicatechin are naturally occurring; however, (–)-epicatechin is widespread in plants in contrast to (+)-epicatechin, which is a minor component of a few species, such as *Camellia sinensis* (tea) (Della Monache et al. 1972). This parallels the uneven occurrence of the enantiomers of catechin. Phytotoxic (–)-catechin and (+)-epicatechin have a 2*S* configuration, suggesting that this feature may be important for phytotoxic activity; the 2,3-trans relationships in (–)-catechin appear to provide additional potency.

Another study was conducted to determine if the hydroxyl groups that confer antioxidant properties to catechin are involved in its phytotoxicity. Pentaacetyl, tetramethoxy, and cyclic derivatives were synthesized and tested for phytotoxicity (Fig. 1B). (±)-Tetramethoxycatechin and (±)-6*a*,12a-*trans*-2,3,8,10-Tetra-hydroxy-

5,5-dimethyl-5,6*a*,7,12*a*-tetrahydro[1]benzopyrano[3,2-*c*], a cyclized derivative, had similar phytotoxic strength to racemic catechin (Veluri et al. 2004). These derivatives also inhibited root differentiation in *C. maculosa*. Although *C. maculosa* is not susceptible to exogenous (–)-catechin in the growth medium, if the flavanol is directly micro-injected into plant cells, mortality occurs (Bais et al. 2003a). Thus, the conversion of (–)-catechin to the much less polar derivatives potentially permits penetration of the phytotoxin into the root, causing growth inhibition at the cellular level. Overall, it was concluded that the antioxidant hydroxyl groups of catechins are not major determining factors for phytotoxicity or antimicrobial activity, but that the ability of a plant to take up extracellular catechin may play a role in its susceptibility (Veluri et al. 2004).

5.2.3 Signal Transduction Studies Provide Clues to Cell Death Response in Susceptible Species

Another clue to the mechanism of (±)-catechin's phytotoxicity was provided by looking at the signaling cascade activated in susceptible plants after treatment with exogenous catechin (Bais et al. 2003a). Both *A. thaliana* and *C. diffusa* showed a transient increase in reactive oxygen species (ROS) and cytoplasmic calcium concentration ($[\mathrm{Ca}^{2+}]_{\mathrm{cyt}}$). Within 10 s of (±)-catechin contact, the roots of these susceptible species generated a burst of ROS that moved back from the root meristematic region and into the central elongation zone, suggesting that these are the first areas affected by the phytotoxin. The spatial kinetics of ROS induction is similar to the pattern of cell death induced by (±)-catechin (Fig. 2B) but occur about 5–10 min before the onset of detectable cell death. A rapid and transient elevation in root tip-localized $[\mathrm{Ca}^{2+}]_{\mathrm{cyt}}$ levels, followed by a decrease in cytoplasmic pH were subsequent to the ROS burst in the susceptible seedlings. When *C. maculosa* was monitored for these responses, there was no detectable increase in cell death (Fig. 2C), ROS, or $[\mathrm{Ca}^{2+}]_{\mathrm{cyt}}$, and the (+)-catechin isomer alone did not induce these responses in any of the plants tested. However, the simultaneous application of an antioxidant, ascorbic acid, in conjunction with (±)-catechin prevented these signaling events from occurring in susceptible plants, and ultimately protected them from the effects of the toxin. These data imply that the phytotoxicity of (±)-catechin is partially due to it's ability to generate free radicals in the plant and that the ROS/$[\mathrm{Ca}^{2+}]_{\mathrm{cyt}}$ signal is an essential prerequisite for (±)-catechin-mediated cell death.

5.2.4 Molecular Tools Provide Clues to Plant Responses to Allelochemicals

While *A. thaliana* is not an ecologically significant model for studying allelopathic interactions between invasive plants and their neighbors, it does provide a useful genetic tool. By testing allelochemicals against *A. thaliana*, it is possible to use microarray analysis to track gene regulation in response to these toxins. Bais et al. (2003a) used this system to analyze global gene expression to define potential transcriptional events associated with (±)-catechin's phytotoxic response. A cluster of 10 genes were upregulated 10 min post (±)-catechin treatment, and included genes associated with a steroid sulfotransferase-like protein, α-cystathionase, a chlorophyll binding protein, calmodulin, a ribosomal protein L9, peroxidase ATP21a, and four genes which did not have any homology with known genes in other organisms. These ten genes may be implicated in plant-specific early signal transduction events linked to oxidative stress. One hour post (±)-catechin treatment, a significant number of genes related to oxidative stress, including glutathione transferase (GST), monooxygenase, lipid transfer protein, heat shock protein (HSP), and blue copper binding protein were upregulated. Many of these same genes were down-regulated 12 h post treatment. Genes coding for enzymes of the phenylpropanoid pathway (*LDOX*) and terpenoid phytoalexin pathway (*VS*) were also among those induced within 1 h of treatment with (±)-catechin. Although metabolites synthesized by the above-mentioned enzymes are mainly involved in defense reactions against pathogens and UV, many of them act as antioxidants as well (Sticher et al. 1997). While this particular study revealed insights into early cellular responses to (±)-catechin, the exact cellular target of catechin is still unknown.

5.3 Mechanisms of Resistance

Understanding how some plants avoid the toxicity of allelochemicals is as important as understanding the mode of action of the allelochemicals themselves. When studying allelopathic invasive plants, this knowledge could help land managers choose resistant species for revegetation and reclamation of invaded sites. It is also important when exploring the use of an allelochemical as an environmentally benign herbicide to determine how quickly target plants may be able to develop resistance, or how soil properties may affect the chemical. Some plants have found ways to reduce the effects of allelochemicals produced by neighboring plants through detoxification mechanisms such as carbohydrate conjugation, sequestration or secretion, and oxidation of the phytotoxic compounds (Inderjit and Duke 2003). Plants equipped to metabolize the benzoxazinone degradation product, benzoxazolin-1 (3*H*)-one (BOA), detoxify this allelopathic compound through *N*-glucosylation, addition of a pentose sugar moiety, or through hydroxylation followed by glucosylation (Weiland et al. 1998, 1999) resulting in several structurally similar but less toxic products such as BOA-6-*O*-glucoside. Detoxification products are then released into the environment via root exudation where they are presumably metabolized by soil microorganisms (Sicker et al. 2001).

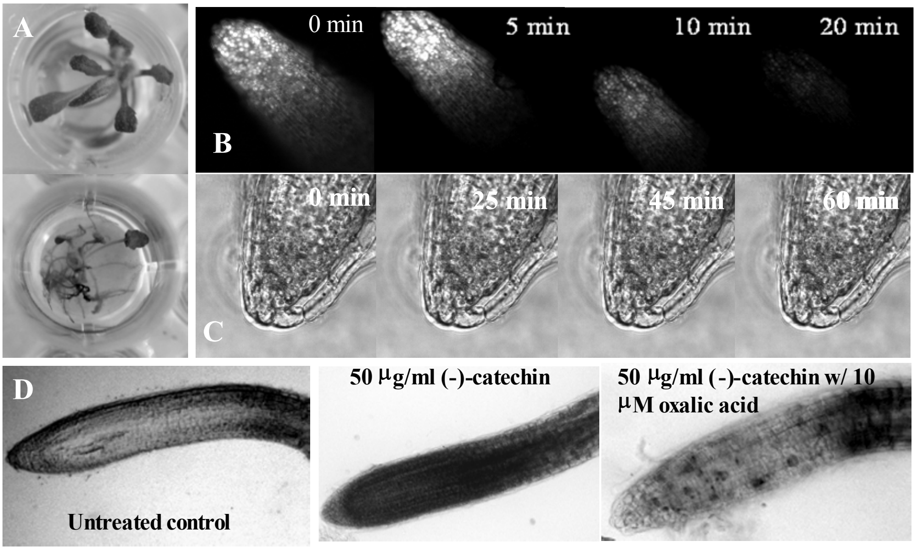

Fig. 2. (**A**) Untreated *A. thaliana* plants remain healthy in in vitro culture, while those treated with 50 g/mL (±)-catechin display mortality after 7 days. (**B**) A fluorescent dye, fluorescein diacetate (FDA), is used to demonstrate the spatial kinetics of cell death in *A. thaliana*. As the cells die there is a progressive loss of fluorescence, demonstrating that cell death in the root tip occurs within 20 min of treatment with 100 g/mL of (±)-catechin. (**C**) *Centaurea maculosa* plants treated with (±)-catechin display no signs of cell death, even after 1 h. (**D**) Reactive oxygen species (ROS) accumulate in *A. thaliana* roots treated with (±)-catechin; however, addition of oxalic acid prevents this ROS accumulation and rescues the roots from cell death

The rhizosphere soil properties and microorganisms may also assist in decreasing the phytotoxicity of allelopathic compounds, particularly phenolic acids. Soil microorganisms rapidly mineralize phenolic compounds because they have a higher energy/weight ratio than simple sugars (Schmidt and Ley 1999). Blum et al. (2000) demonstrated that soil rich in individual phenolic acids induced colonization by phenolic acid utilizing bacteria, and that these bacteria reduced the seedling growth inhibition normally attributed to phenolic acids. Phenolic acids also react with soil particles via sorption and oxidation, decreasing the phytotoxicity of these compounds (Ohno and First 1998; Ohno 2001).

5.3.1 Some Plants Display Natural Resistance to (±)-Catechin

Phytotoxicity studies with (±)-catechin revealed that different plant species showed varying susceptibility to the phytotoxin (Weir et al. 2003; Perry et al. 2005a). The North American natives, *Gaillardia grandiflora* and *Lupinus sericeus*, showed resistance to (±)-catechin in vitro and to *C. maculosa* invasions in field sites (Ridenour and Callaway 2001; Callaway et al. 2004). It was discovered that root exudates of both plant species contained high levels of oxalic acid relative to susceptible plant species examined, and that secretion was increased after elicitation with (±)-catechin (Weir et al. 2006). Exogenous application of oxalic acid in conjunction with phytotoxic concentrations of (±)-catechin prevented phytotoxicity, accumulation of ROS, and decreased membrane lipid peroxidation in *A. thaliana* seedlings (Fig. 2D) (Weir et al. 2006). Oxalic acid is a known antioxidant and iron chelator (Kayashima and Katayama 2002), suggesting that plants that can mount a sufficient antioxidant response could avoid phytotoxicity by preventing free radical formation and initiation of the cell death signalling cascade described by Bais et al. (2003a).

There is also evidence that some native grass species are evolving resistance to (±)-catechin. Invasion by exotic plants, such as *C. maculosa*, that cause extensive mortality in native populations can be a driving selective force on native species. Callaway et al. (2005) found that individuals from some native grass populations, which survived extended invasion by *C. maculosa*, were more resistant to the general effects of the weed and to its root-secreted toxin than individuals from populations that had not experienced invasion. Native species grown from the seed of these surviving individuals were also more resistant to *C. maculosa*, its root exudates, and to isolated (±)-catechin, than conspecifics grown from naïve native seed. This suggests that allelochemistry may drive native plants to evolve resistance to allelotoxins and may explain why many invasive plants are not as successful in their native habitats, supporting the "novel weapons hypothesis".

5.4 Future Directions

While much progress has been made in determining how (±)-catechin assists *C. maculosa* in its interactions with neighboring plants, there are still many mysteries to unravel. Ecological studies to determine the distribution and persistence of (±)-catechin in the soil, as well as the ability of neighbors to respond or adapt to it, need to be refined to increase our understanding of how this chemical could influence community structure. Alternative physiological roles for racemic catechin should also be explored. Artificially buried *C. maculosa* seeds showed an average in situ germination rate of 51% after eight years, while 90% of the ungerminated seeds that were recovered remained viable (Davis et al. 1993). Davis et al. (1993) hypothesized that this slow germination may be influenced by the availability of water for imbibition; however, it is possible that *C. maculosa* produces catechin as a mechanism to modulate its own growth or germination to reduce intraspecific competition. Observed spatial segregation of mature *C. maculosa* plants could indicate that successful germination requires soil where less (±)-catechin is present. Furthermore, in garden experiments, a large percentage (up to 93%) of *C. maculosa* plants bolt after one year (Schirman 1981); however, experiments done in natural *C. maculosa* populations showed none of the first year plants bolting, and only 10% of the two-year-old plants bolted (Story et al. 2001). This delayed maturation observed under natural field conditions may also be an effect of (±)-catechin present in the soil of a well-established *C. maculosa* site that does not occur under controlled conditions where there would be no residual (±)-catechin in the soil.

Further biochemical and molecular evaluation will help clarify the exact cellular mechanism of (±)-catechin toxicity and the gene cascades involved in these responses. This knowledge may eventually be utilized to engineer plants/crops that are resistant to the allelochemicals produced by invasive weeds. In addition, elucidation and manipulation of the catechin biosynthetic pathway could be useful in clarifying the impact of allelopathy versus competition in *C. maculosa* invasion ecology.

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6. Allelopathic Mechanisms and Experimental Methodology

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Abstract. Allelopathy is a complex ecological phenomenon, and it has proven notoriously difficult to develop experimental methods that can distinguish the role of both chemical interference and resource competition in plant growth. This paper discusses two promising new methodologies which can be used to study allelopathic interactions in the greenhouse and field. (a) Bioassays in which the density of the susceptible plant species is varied give results contrary to the expected results of resource competition when a toxin is present in the soil. Compared to a control soil, growth reductions will occur at low density but diminish or disappear at high density. Furthermore, individual plant size may actually increase as density increases. These density-dependent phytotoxic effects result from the fact that plants growing at low densities have a larger amount of the toxin available per plant, and therefore suffer greater growth reductions than those in high densities, where each plant receives a proportionately smaller dose of the toxin. (b) Sorbents based on the polymer polydimethyl-siloxane (PDMS) show promise for the measurement of allelochemical fluxes in the rhizosphere. Various forms of PDMS have been demonstrated to pick up increasing amounts of the lipophilic root exudate sorgoleone when buried beneath sorghum-sudangrass hybrid plants. Work is continuing to determine the stability of sorbed compounds, how broad a range of compounds can be effectively trapped by PDMS, and what forms of PDMS are most useful for field studies.

6.1 Introduction

6.1.1 Allelopathy – a Complex Ecological Phenomenon

Molisch (1937) defined allelopathy as encompassing both stimulatory and inhibitory biochemical interactions among plants at all levels of complexity, including microorganisms. In subsequent years, there has been recognition that allelopathy may involve more than direct chemical effects of one plant on a neighbor. Effects may be indirect, mediated by microbial or non-microbial degradation of a plant compound.

Fig. 1. The characteristic bare zone around the Florida rosemary *Ceratiola ericoides* is seen here along a roadside border. The bare zone here is approximately 1 m in extent.

Ceratiola ericoides Michx. is one of several perennial shrubs in the Florida scrub community found to inhibit the germination and growth of grasses and herbs (Fig. 1). Aqueous leaf washes of *Ceratiola* contain the inactive dihydro-chalcone ceratiolin, which degrades in the presence of sunlight to phytotoxic hydrocinnamic acid (Tanrisever et al. 1987; Fischer et al. 1994). The secondary chemistry of *Polygonella myriophylla* (Small) Horton, another of the allelopathic scrub perennials, is dominated by glycosides of hydroquinone and gallic acid.

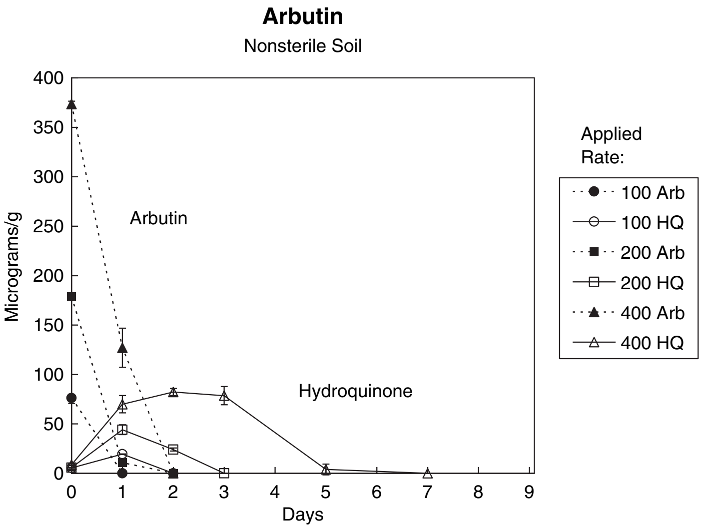

Fig. 2. Degradation of exogenously applied arbutin (Arb) in nonsterile soil from beneath *Polygonella myriophylla*. Dashed lines show the disappearance of arbutin, while solid lines show the appearance and disappearance of hydroquinone (HQ), which was formed by microbial degradation of its glycoside arbutin. In sterile soil, arbutin was stable over the time period of the experiment. Bars indicate standard error. If not shown, standard errors are too small to depict visually (original figure appeared in Weidenhamer and Romeo 2004; used with permission of Springer Science and Business Media)

Microorganisms in scrub soils have been shown to convert the hydroquinone glycoside arbutin to hydroquinone and then to benzoquinone (Figs. 2 and 3). It is hypothesized that these compounds are the active allelopathic agents of this plant (Weidenhamer and Romeo 2004). Plant allelochemicals may exert effects indirectly by their impact on the soil environment. *Pinus muricata* leaf litter releases polyphenols that complex Al thereby increasing its toxicity (Northup et al. 1999). Another example is the apparent inhibition of black spruce regeneration by *Kalmia angustifolia* in Canadian boreal forests, which is hypothesized to result from the interaction of several factors including the impact of Kalmia phenolics on soil nutrient balance (Mallik 2001; Inderjit and Mallik 2002).

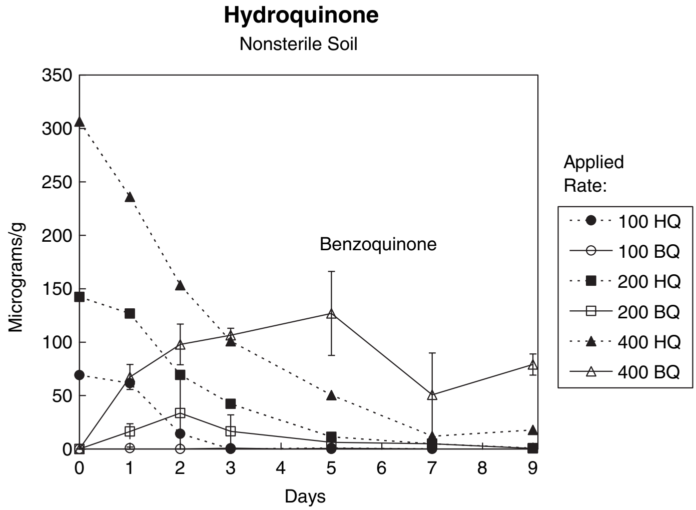

Fig. 3. Degradation of exogenously applied hydroquinone (HQ) in nonsterile soil from beneath *Polygonella myriophylla*. Dashed lines show the disappearance of hydroquinone, while solid lines show the appearance and disappearance of benzoquinone (BQ), which was formed by microbial degradation of hydroquinone. In sterile soil, hydroquinone was stable over the time period of the experiment and amounts of benzoquinone found were <5 mcg/g. Bars indicate standard error. If not shown, standard errors are too small to depict visually (original figure appeared in Weidenhamer and Romeo 2004; used with permission of Springer Science and Business Media)

Allelopathic effects may also be mediated by inhibition of symbiotic *Rhizobium* spp. or mycorrhizae (Brown and Mikola 1974; Nilsson et al. 1993). In certain cases, allelopathic effects may be mediated by a third organism. An example would be a plant that stimulates the growth of microorganisms which produce phytotoxins that inhibit a neighboring plant. Kaminsky (1981) provided evidence that the toxic effects of the chaparral shrub *Adenostoma fasciculatum* were produced by associated microorganisms rather than the shrub itself.

Further complicating the picture is the fact that allelopathy can and will interact with resource competition and abiotic factors on plant growth. For example, nutrient limitation increases the toxicity of some allelochemicals, and can increase their production. Williamson et al. (1992) found that hydrocinnamic acid was more toxic to the grass *Schizachyrium scoparium* in low N and low K conditions. Thus, competition for nutrients might result in allelopathic inhibition (Blum et al. 1999). The toxicity of phenolic acids is influenced by factors such as soil pH (Blum 1995). Allelopathic inhibition will likely reduce plant effectiveness in competing for resources (Humphry et al. 2001). The results of Nilsson (1994) show strong interaction of resource competition and allelopathy for crowberry (*Empetrum hermaphroditum*). Scots pine (*Pinus sylvestris* L.) was grown with crowberry using PVC pipes to reduce root competition and activated carbon to reduce allelopathy. The greatest inhibition occurred when pines were subject to both allelopathy and resource competition, while the effects of either allelopathy or belowground competition caused intermediate inhibition. The recent recognition that plant secondary chemistry can be influenced by herbivory (e.g. Baldwin and Schultz 1983) suggests that allelopathic effects of a plant might be intensified by herbivores. This possibility is supported by the results of Callaway et al. (1999), who found that herbivory of *Centaurea maculosa* in the field by a biocontrol moth resulted in reduced growth of competing *Festuca idahoensis*. Similar results were observed in a greenhouse study with another herbivore. They hypothesized that moderate herbivory might induce the production of defensive chemicals that had allelopathic effects. Thelen et al. (2005) demonstrated that experimental attacks on *C. maculosa* by either root boring biocontrol insects or a parasitic fungus both increased catechin exudation and allelopathic effects on susceptible native plants.

Beyond the possible direct and indirect allelopathic effects of one plant on another, there is growing recognition that allelopathy may affect community-level processes such as succession, nitrogen cycling and community dynamics (Muller 1966; Rice 1984; White 1994; Wardle et al. 1998; Inderjit and Weiner 2001).

6.1.2 Methodological Consequences

The complexities of allelopathic interactions have been explored in several reviews (Einhellig 1987; Williamson 1990; Weidenhamer 1996; Inderjit and del Moral 1997; Blum et al. 1999), and have profound implications for experimental design (Romeo and Weidenhamer 1998; Blum 1999; Weidenhamer 2006). Romeo and Weidenhamer (1998) note several important guidelines, including: (a) The biology of the interaction should be studied for clues as to possible mechanisms. (b) Bioassays must be conducted with associated plant species to be ecologically meaningful. (c) The possible role of environmental factors cannot be neglected in designing appropriate bioassays. (d) The active compound(s) may not be in the same chemical form found in the plant. (e) Bioassays should reflect natural conditions with realistic toxin concentrations that approximate those produced in the natural system. These are discussed in more detail later in this review. Unfortunately, much work reported in the scientific literature has failed to take this ecological complexity into account (Romeo 2000).

6.1.3 The Challenge: Distinguishing Allelopathy from Other Factors Affecting Plant Growth

The focus of this review is specifically the problem of distinguishing allelopathy from other mechanisms of plant-plant interaction, as well as from abiotic factors (e.g. soil pH) that may impact plant growth. Such efforts are complicated by the fact that plants experience their physical, chemical and biological environment all at once, and various mechanisms of plant–plant interaction may and do occur simultaneously, sequentially, or interact with one another. While some have argued that this complexity makes it impossible to separate allelopathy from resource competition (Inderjit and del Moral 1997), I will argue that it is possible to devise bioassays that in the presence of phytotoxic concentrations of allelochemicals yield results inexplicable solely on the basis of resource competition. Indeed, investigators must be able to identify positive evidence that differentiates allelopathy from other mechanisms of plant-plant interaction if allelopathy is to be a scientifically viable hypothesis.

6.2 Density-Dependent Phytotoxicity

6.2.1 Density and Plant Growth

The biomass of plants is a function of density. When plants are grown together, total yield (which may be measured as production of fruit or seed, or total biomass) will increase linearly with density up to the point at which neighboring plants begin to compete with one another for resources (Fig. 4a). Above this density yield per unit area remains constant across a wide range of densities (Kira et al. 1953). In the range of densities where yield is constant, individual plant biomass decreases as plant density increases. The relationship of log mean plant mass and log density is linear with a slope of –1 (Fig. 4b). While factors such as resource availability may alter the maximum yield achieved under given conditions, and thus the intercept of the log mean mass–log density line, they do not alter the predicted slope. In the context of this discussion of ways to distinguish allelopathy and resource competition, it needs to be realized that the cause of the decrease in plant size with increasing plant density is resource competition. The universality of the –1 law of constant final yield (White and Harper 1970; Harper 1977; Gorham 1979; White 1980) has resulted in it being considered one of only two major 'laws' in plant ecology (White 1980; Harper 1982).

It is worth noting the –1 line (Fig. 4b) represents an endpoint in a time course that begins at planting with a slope of zero, when all seedlings have approximately the same mass and the log mean plant mass–log density line is horizontal. The line will become inclined as the plants grow and begin to compete, first at the highest densities, and gradually reaches a slope of –1 (Harper 1977).

6.2.2 What is Density-Dependent Phytotoxicity?

Density-dependent phytotoxic effects may be defined as the differences in the magnitude of inhibition observed when plants grow at varied densities in soil containing a phytotoxic substance (Weidenhamer et al. 1989). Density-dependent phytotoxicity results from the fact that plants "compete" for phytotoxins in the same way that they do for resources. While the consequences of winning competition for resources are positive, the consequences of winning the competition for phytotoxins are negative. This differential in response thus provides a means to distinguish the effects of resource competition from those of allelopathy. Hoffman and Lavy (1978) demonstrated that plants compete for herbicides with experiments using ^{14}C-labelled atrazine, in which atrazine uptake per plant decreased by 50% when soybean populations increased from one to six plants per pot.

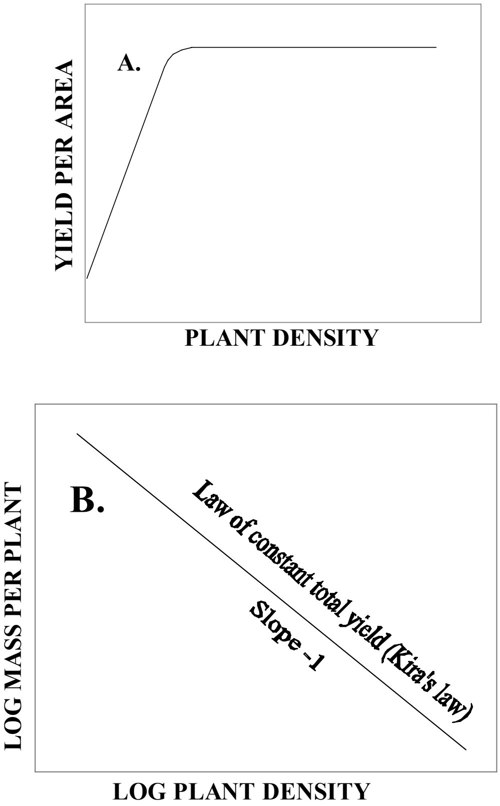

Fig. 4. Expected response of plant growth to density: (**A**) expected response of yield to increasing plant densities; (**B**) expected relationship of log mean mass per plant and log plant density in the range of plant densities where total yield is constant (original figures appeared in Weidenhamer et al. 1989; used with permission of Blackwell Science, Ltd.)

An alternate way of understanding the basis for density-dependent phytotoxic effects is to realize that in a given soil volume containing a finite amount of phytotoxin, plants growing at low densities have a larger amount of the toxin available per plant and therefore should suffer greater growth reductions than those in high densities, where the toxin is shared ('diluted') among many plants and each receives a proportionately smaller dose. This contrasts with resource competition, where plants growing at low densities have a larger amount of the resource available per plant and therefore have increased growth as a result. This may lead to results contrary to the expected results of resource competition: (a) Compared to a control soil, growth reductions occur at low but diminish or disappear at high density; (b) Individual plant size may actually increase as density increases. The results of adding activated carbon to the soil (Nilsson 1994; Ridenour and Callaway 2001) and increasing plant density are similar in reducing the amount of toxin available to individual plants.

These techniques are therefore complementary approaches to distinguishing resource competition and allelopathy.

6.2.3 Consequences of Density-Dependent Phytotoxicity

Density-dependent phytotoxicity was first reported in work with herbicides (Skipper 1966; Hoffman and Lavy 1978; Andersen 1981; Winkle et al. 1981). Weidenhamer et al. (1987, 1989) reported the first investigations of the density-dependent effects of natural allelochemicals. Weidenhamer et al. (1989) grew bahiagrass (*Paspalum notatum*) in soil treated with hydroquinone and gallic acid, the suspected inhibitors from *Polygonella myriophylla*. At 8 weeks, shoot biomass of bahiagrass grown in soil treated with 400 μg/g of each compound was 63% of the corresponding control at a density of two seedlings per pot, but there was no inhibition in plants with 16 seedlings per pot. Stimulation of bahiagrass growth was observed at lower rates, and was also density-dependent. In another experiment, tomato (*Lycopersicon esculentum* Mill.) was grown in flats of soil collected from beneath black walnut (*Juglans nigra* L.) and adjacent fields. Growth reductions of tomato were much greater in the low density treatment.

Thijs et al. (1994) grew corn and soybean together using a target-neighbor design, in which varying densities of neighbors are grown around a central target plant. The broadleaf herbicide atrazine, which is highly phytotoxic to soybean but not to corn, was then applied to the pots. At the highest applied rate of atrazine, dry mass of target soybean plants increased by 150% (from 0.2 to 0.5 g) as the number of corn neighbors increased from 0 to 9–12 per pot. Such a result is contrary to the expected results if resource competition were the major interaction between the corn and soybean plants. However, the results are understandable on the basis that competition for atrazine by the corn neighbors reduced the amount of herbicide available to the soybean plants.

6.2.4 A Growing Body of Evidence

Tseng et al. (2003) incorporated leaf powder of *Macaranga tanarius* (L.) Muell.-Arg., a suspected allelopathic species, into soil. Lettuce proved to be sensitive to *Macaranga*, and growth reductions of lettuce diminished compared to a corresponding control as lettuce density increased. Gentle and Duggin (1997) have carried out field experiments to investigate the allelopathic potential of *Lantana camara* L. toward tree species. They planted two trees, *Cryptocarya rigida* and *Alectryon subcinereus*, at densities of 10, 20 and 30 seedlings per m^2 in plots where the *Lantana* was either removed, burned, or left in place. Where *Lantana* was removed, the biomass of tree seedlings decreased as the seedling density increased. However, were *Lantana* was left in place, the biomass of tree seedlings actually increased with increasing density. An increase in individual plant biomass with increasing plant density is reflected by a reversal in the slope of the log biomass – log density line (Fig. 5).

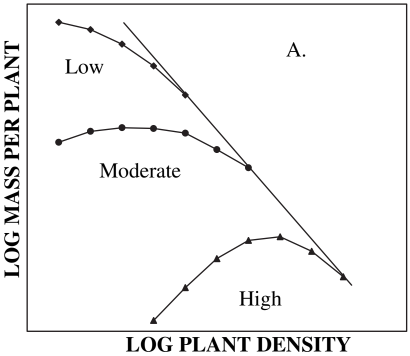

Fig. 5. Effect of phytotoxins on yield–density relationships. Graph shows the predicted deviations in the log mean mass–log density relationship in the presence of low, moderate and high concentrations of phytotoxins (original figure appeared in Weidenhamer et al. 1989; used with permission of Blackwell Science, Ltd.)

6.2.5 Practical Guidelines for Bioassays

The question confronting the researcher confronted with a suspected allelopathic interaction is where to begin in designing experiments. As noted earlier, allelopathy is a complex ecological phenomenon, and operates in concert with resource competition and environmental factors to impact plant growth. This complexity must be taken into account in designing studies to elucidate suspected allelopathic interactions. And while not all allelopathic effects are direct effects of one plant on another (Inderjit and Weiner 2001), there *are* numerous examples of direct plant–plant effects, and these will continue to be of interest to researchers looking for ecological means of weed control in agricultural systems.

Though the ecological complexity of allelopathy makes the development of straightforward, standard protocols to apply in the case of a suspected allelopathic interaction unlikely, there are a number of useful guidelines that should be taken into account (Romeo and Weidenhamer 1998). The list below is not intended to be comprehensive, but rather to be useful checkpoints for researchers just starting out in their investigations.

Guideline 1: The biology of the interaction needs to be understood as much as possible. If plant growth is being suppressed by inhibition of mycorrhizal or *Rhizobius* spp. symbionts, for example, this will suggest very different bioassay strategies than might otherwise be selected.

Guideline 2: It is crucial that bioassays be conducted with associated plant species if the results are to provide insight. Screening cultivars of rice for toxicity to lettuce seedlings may provide leads on new phytotoxic chemicals, but does not provide insight as to whether these cultivars are toxic toward the common weeds of rice fields. The literature on allelopathy is filled with numerous examples of species-specific differences in sensitivity to allelochemicals. The best way to address this issue is to perform bioassays with the target species of interest.

Guideline 3: The possible role of environmental factors cannot be neglected in designing appropriate bioassays. For example, if conditions in the field are hot, regularly exceeding 35°C, the investigator must question whether bioassays at room temperature (20–25°C in an air-conditioned laboratory) provide the best information, given that temperature is known to affect both the toxicity of allelochemicals and the rates of microbial degradation processes.

Guideline 4: The active compound(s) may not be in the same chemical form found in the plant. The importance of environmental and microbial processes in activating relatively non-toxic allelochemicals in Florida scrub plants implies that the use of bioassay-guided fractionation of crude plant extracts may be misleading when environmental transformation of plant allelochemicals is significant (Weidenhamer and Romeo 2004).

Guideline 5: Bioassays should reflect natural conditions with realistic toxin concentrations that approximate those produced in the natural system. All too often I have reviewed papers in which an investigator has prepared a concentrated extract of plant foliage and tested it for effects on germination against common assay species, sometimes without even running controls for osmotic effects. The meaning of such assays is dubious. Zackrisson and Nilsson (1992) showed that a brief exposure to snow melt (presumably containing allelochemicals from *Empetrum hermaphroditum*) was all that was needed to significantly reduce the growth of pine. Such an assay provides much more compelling evidence of allelopathy because it uses ecologically realistic concentrations.

Guideline 6: It is important not to overlook alternative explanations for what appear to be allelopathic effects. Studies by Gliessman and Muller (1972, 1978) and by Putnam and colleagues (Barnes and Putnam 1983, 1986; Putnam et al. 1983) provide good examples of how the contribution of factors such as shading, soil moisture, and so on can be determined and eliminated as explanations for presumed allelopathic effects.

Guideline 7: The most difficult alternative explanation to rule out is generally competition for water or nutrients by the suspected allelopathic plant. In this review, I have argued that demonstrating the presence of density-dependent phytotoxic effects is one means of obtaining evidence that allelopathy may be operative in a particular field or laboratory situation. In bioassays, the greatest inhibition will be seen when using the lowest density of assay species and largest pot volumes (Weidenhamer et al. 1987; Romeo and Weidenhamer 1998). Density-dependent phytotoxic effects are most likely to be manifested as assay species density changes from a single plant (i.e. no competition for phytotoxins) to 2, 4, 8 and 16 plants per unit area or per pot. In the case of assays involving a suspected allelopathic plant and a sensitive target, it should be remembered that increasing the density of the source plant will also increase the amount of allelochemicals to the target. Density-dependent phytotoxic effects will be manifested by differences in growth of the sensitive target when the target is grown over a range of densities. The use of activated carbon (Nilsson 1994) is an alternate technique that can be used to distinguish the separate contributions of allelopathy and competition to the inhibition of plant growth. One of the potential difficulties with this technique is that the addition of activated carbon to soil can affect plant growth, thereby potentially confounding detection of allelopathic effects. Density-dependent phytotoxic effects provide an independent means of ruling out competition for nutrients and water as the explanation for apparent allelopathic effects.

6.3 Chemical Investigations of Allelopathic Interactions

No discussion of experimental methodology for the study of allelopathy would be complete without consideration of the chemistry of allelopathic interactions. Our knowledge of the chemical compounds responsible for allelopathic effects has grown remarkably in recent years. Several allelochemicals with toxicities rivaling synthetic herbicides are known, including α-terthienyl (Campbell et al. 1982), artemisinin (Duke et al. 1987) and sorgoleone (Nimbal et al. 1996). However, in almost every case, very little is known of the dynamics of these compounds in soil. The need for quantitative information on putative toxins has been emphasized repeatedly. Fuerst and Putnam (1983) asserted that quantifying the amount of toxin released to the environment and taken up by the target plant was crucial to proving a hypothesis of allelopathy, a position that has been echoed by other workers (Radosevich and Holt 1984; Willis 1985).

Existing methods to measure allelochemical concentrations in soil typically rely on aqueous or solvent extractions (e.g. Dalton et al. 1987; Ponder and Tadros 1985) or direct analysis of soil solution (e.g. Gallet and Pellissier 1997). The low concentrations of allelochemicals typically found in soils, and the often rapid microbial degradation of allelochemicals, are cited as evidence that these compounds do not play a significant role in plant–plant interactions (Schmidt 1988). However, the key question is not how much phytotoxin is present at any one point in time, but the flux rate over time (Williamson and Weidenhamer 1990). Static concentrations in soil reflect the balance of input vs. output rates for a compound. Moreover, static concentrations provide no information about input and output rates, any more than static concentrations of ammonium and nitrate in the soil provide information about the magnitude of the processes of nitrogen mineralization and denitrification occurring in that soil. Few attempts have been made to quantify input and output rates of suspected allelopathic compounds.

Recently, it has been shown that polydimethylsiloxane-based materials may have potential as sorbents to provide information on allelochemical dynamics in soil (Weidenhamer 2005). These materials are widely used by environmental scientists to monitor trace levels of anthropogenic pollutants (Pawliszyn 1999; Baltussen et al. 1999, 2002; Mayer et al. 2000; Popp et al. 2003). They are also being applied to the analysis of natural samples such as direct analysis of fruit by insertion into strawberries (Kreck et al. 2001), and analysis of volatiles emitted by living plants (Vercammen et al. 2000).

The suitability of PDMS sorbents to monitor allelochemicals in soil was demonstrated using stir bars coated with PDMS (stir bar sorptive extraction), technical grade optical fiber coated with a thin film of PDMS (matrix-solid phase microextraction), and PDMS tubing to monitor the amounts of sorgoleone beneath greenhouse-grown sorghum-sudangrass hybrid plants (*Sorghum bicolor* x *S. sudanense*) over a 3 months period (Weidenhamer 2005). PDMS probes were removed at 29, 55 and 88 days after planting. As analyzed by HPLC, the amount of sorgoleone recovered increased with time. The strong performance of PDMS tubing and PDMS-coated optical fiber is noteworthy also because both materials are less expensive than the commercially available PDMS-coated stir bars. Because of its rigidity, optical fiber can be directly inserted into the soil with minimal disturbance. PDMS tubing could either be buried or also inserted directly into the soil if a stiff wire is inserted into the tubing. These materials are therefore potentially useful for non-destructive sampling of the rhizosphere in large-scale greenhouse and field studies.

Further work is ongoing to elucidate the stability of sorbed compounds over time, how broad a range of compounds can be effectively trapped by these sorbents, and what forms of PDMS are most advantageous for field studies. The demonstration that fluxes of allelochemicals can be measured in the rhizosphere will not prove that allelopathic interactions are occurring. However, the demonstration of allelopathic interactions without data on allelochemical dynamics in soil will remain problematic. PDMS-based materials provide a new tool for obtaining this information, and thereby helping to assess the importance of allelopathic processes in plant communities.

6.4 Summary

Allelopathy has been implicated as a causal factor in vegetation patterning and ecological processes such as nitrogen fixation, and understanding allelopathy may hold the key to new weed control practices in agriculture. Devising experiments which can distinguish the impact of both allelopathy and resource competition on plant growth has been a major challenge both from the standpoint of bioassays and the measurement of allelochemical dynamics in the soil. Given the complexity of allelopathy as an ecological phenomenon, no one experiment can be expected to demonstrate allelopathic mechanisms are operative in a particular field situation. However, new methodologies have been developed for both bioassays and chemical analysis of the rhizosphere which may help overcome some of the experimental problems that have hindered the study of allelopathic interactions.

Bioassays in which the density of the susceptible plant species is varied are proposed as a means to identify situations in which allelopathy may be operative. Density-dependent phytotoxic effects are defined as the differences in the magnitude of inhibition observed when plants grow at varied densities in soil containing a phytotoxic substance. In a given soil volume containing a finite amount of phytotoxin, plants growing at low densities have a larger amount of the toxin available per plant, and therefore suffer greater growth reductions than those in high densities, where each plant receives a proportionately smaller dose of the toxin. This leads to results contrary to the expected results of resource competition: (a) Compared to a control soil, growth reductions occur at low but diminish or disappear at high density; (b) Individual plant size may actually *increase* as density increases. A growing literature now supports the usefulness of experiments varying plant density as a tool to distinguish allelopathy and resource competition.

Sorbents based on the polymer polydimethylsiloxane (PDMS) have been shown to pick up increasing amounts of the lipophilic root exudates sorgoleone when buried beneath sorghum-sudangrass hybrid plants. Work is in progress to elucidate the stability of sorbed compounds over time, how broad a range of compounds can be effectively trapped by these sorbents, and what forms of PDMS are most advantageous for field studies. PDMS sorbents hold promise for moving beyond the measurement of static concentrations of allelochemicals in soil to the measurement of allelochemical dynamics over time.

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7. Indirect Effects of Phenolics on Plant Performance by Altering Nitrogen Cycling: Another Mechanism of Plant–Plant Negative Interactions

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Abstract. Negative interactions among plants have been explained by two main mechanisms, competition and allelopathy. Here, I focus on a third mechanism resulting from the interaction of the previous two, and based upon changes in nutrient availability caused by the release of phenolic compounds into the soil. Phenolic compounds globally decrease soil N availability by changing microbial activity. The relevance of these processes in natural conditions, and the consequences that changes in N availability might have on the distribution of plant species in the ecosystem, remains to be evaluated. Here I describe the specific mechanisms by which phenolics change soil N cycling and the factors that might alter the fate and role of phenolics in the ecosystem. I review five examples in which species with high concentrations of phenolic compounds known to interfere with growth of other plants (*Cistus albidus*, *Ledum palustre*, *Empetrum hermaphroditum*, *Populus balsamifera* and *Kalmia angustifolia*) decrease N availability in natural conditions. In those studies, phenolics do not affect N cycling in natural systems by forming complexes with proteins, as traditionally stated, but by increasing microbial activity after being degraded by microorganisms. The presence of phenolics in plants could be a result of a selective pressure in situations where changing soil chemical properties increase plant competitive ability.

7.1 Introduction

The interference that a particular plant species may have on the growth and development of another has been explained by competition for resources (Grace and Tilman 1990) and allelopathy (Einhellig 1995; Rice 1984). In contrast to competition, the occurrence of allelopathy in natural conditions has not been widely demonstrated mainly due to the small number of field studies and the methodological challenges in linking laboratory bioassays and experiments with field conditions (Inderjit and Callaway 2003; Wardle et al. 1998). Although many compounds have been identified as potential allelopathical agents in laboratory conditions by demonstrating inhibition in seed germination (Ballester et al. 1982; Chaves and Escudero 1997; Pellissier 1993; Zackrisson and Nilsson 1992), root elongation (Ridenour and Callaway 2001), seedling establishment (Nilsson and Zackrisson 1992) and plant growth (Gallet 1994; Zhu and Mallik 1994; Inderjit 1996), the relevance of these processes in natural systems remains controversial.

One problem in identifying allelopathy in natural conditions is that plant secondary metabolites released from foliage and decomposing litter can also promote physical, chemical and biological changes in the soil and thus modify nutrient availability (Bloom and Mallik 2004; Hättenschwiler and Vitousek 2000; Wardle et al. 1998). When this happens, identifying the specific factor affecting plant performance can be very difficult (Inderjit and Del Moral 1997). Some studies have specifically addressed the issue of separating allelopathy from below-ground competition (Callaway and Aschehoug 2000; Nilsson 1994; Ridenour and Callaway 2001; Weidenhamer et al. 1989) but this objective has not been always accomplished. As an example, Nilsson (1994) designed an experiment in which the phenolic-producer *Empetrum hermaprhoditum* was physically separated from its target (*Pinus sylvestris*) to exclude root competition, and chemically separated by adding activated charcoal to the soil, which absorbed the secondary metabolites released by leaching, to exclude allelopathy. Because both physical and chemical exclusions were found to positively affect *P. sylvestris* growth, the author concluded that competition and allelopathy were simultaneously occurring (Nilsson 1994). However, preventing the secondary metabolites to reach the soil does not only avoid the direct toxic effects on plant physiology, but also any indirect effects that these metabolites can cause on plant growth through changing soil nutrient availability. Because the specific mechanisms of negative interactions between plants are normally not elucidated, the question of whether secondary metabolites are affecting target species directly, or indirectly through the soil, or both, remains open (Michelsen et al. 1995; Wardle and Nilsson 1997). To ascertain the relevance of secondary compounds on natural conditions all effects of allelochemicals on vegetation, including indirect ones, have to be determined. Moreover, these indirect effects could be more important than direct ones in plant population dynamics (Inderjit and Weiner 2001).

Traditionally, the indirect effects of allelochemicals on plant growth through changes in soil processes, also called "soil nutrient imbalance" by Mallik (2003), have been neglected in plant–plant interaction studies or misinterpreted as direct allelopathic effects. Indeed, whether the indirect negative interference among plants through changes in the soil can be considered allelopathy or not is still controversial in the literature (Inderjit and Weiner 2001; Pellissier 1998; Watkinson 1998). Any effect on plant growth caused by secondary compounds has frequently been labeled as "allelopathic" regardless the mechanisms involved (Wardle et al. 1998). This confusion in partially created by the large array of definitions on allelopathy (Mallik 2005), which can include from "any direct or indirect effects by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment (Rice 1984)", to more narrow definitions where only direct effects caused by secondary metabolites are considered (Mallik 2005). The high diversity on allelopathy definitions has probably obscured the role of secondary compounds on controlling plant population dynamics by changes in the soil. To avoid further misinterpretations, I propose to use the term allelopathy exclusively for those processes where direct effects of secondary metabolites on plant physiology are involved (i.e. germination, root elongation, etc.), and regard the indirect effects of allelochemicals on target species through changes in soil nutrient availability as a separate entity from allelopathy and resource competition.

In this chapter I review the effects of phenolic compounds on soil N cycling and discuss how relevant these processes might be in determining negative interactions among plants in natural conditions. Nitrogen is an important limiting factor for primary production in many ecosystems (Vitousek and Howarth 1991) and changes in fluxes of N, especially those related to the production and consumption of NH_4^+ and NO_3^- may have a strong effect in N uptake by plants and lead to changes in species distributions at the ecosystem level. Phenolic compounds are good candidates to interact with N cycling because they have a great capacity to bind organic compounds (Hättenschwiler and Vitousek 2000; Kuiters 1990; Northup et al. 1998; Waterman and Mole 1994). Due to its high solubility, phenolics are easily leached by rainfall from green foliage and decomposing litter reaching the soil underneath the canopy (Castells et al. 2003; Gallet and Pellissier 1997; Harborne 1997; Inderjit and Mallik 1996a; Kuiters and Sarink 1986) and eventually accumulating over time (MacKenzie et al. 2004). The overall effect of phenolics on soil N cycling is a decrease in the availability of inorganic N. By creating an unfavorable environment phenolic compounds can indirectly affect target species performance and confer a competitive advantage to the phenolic producer. The ecological relevance of phenolic compounds is likely to be most important in ecosystems where slow-growing, N-limited species with high presence of phenolics predominate, such as Mediterranean and Boreal ecosystems.

7.2 Interactions of Phenolic Compounds with N Cycling

Plant phenolic compounds leached to the soil are expected to have a dominant role in controlling many aspects of plant–soil interaction by either inhibiting or enhancing microbial activity (Kuiters 1990; Northup et al. 1998; Schimel et al. 1996). Phenolics globally decrease soil N availability by slowing decomposition, decreasing N mineralization, enhancing N immobilization, inhibiting nitrification and N-fixation, and modifying mycorrhizal fungal activity, as detailed below. These effects do not exclude each other, but, in general, the specific chemical properties of a given phenolic compound determine its major type of interaction with the N cycling. For instance, low molecular weight phenolics, such as phenolic acids, are more easily degraded by microbes and are related to increases in N immobilization, while more complex phenolics such as condensed tannins form complexes with proteins, including extracellular enzymes present in the soil, which may slow decomposition or N mineralization (Fierer et al. 2001; Hättenschwiler and Vitousek 2000).

7.2.1 Decomposition

Decomposition rates are determined by litter chemical traits such as C:N and lignin:N ratio; however concentration of phenolic compounds also have a significant role (Hättenschwiler and Vitousek 2000; Hobbie 1992). One of the most characteristic properties of phenolic compounds is their capacity to form recalcitrant complexes with proteins (Fierer et al. 2001; Hättenschwiler and Vitousek 2000; Kuiters 1990).

These complexes originate during senescence when phenolics stored in vacuoles come into contact with cytoplasmatic proteins or directly in the soil when phenolics are leached from foliage (Hättenschwiler and Vitousek 2000). The protein–phenolic complexes are difficult to degrade and they can stabilize more than 60% of foliar N (Kuiters 1990). Microbial flora involved in litter decomposition can be inhibited by phenolics when the exoenzymes released to the soil are inactivated by the formation of phenolic–protein complexes or when substrates required for microbial growth are depleted (Harrison 1971; Kraus et al. 2003). Thus, phenolic concentrations in foliage have been correlated with slower soil organic matter decomposition and turnover rates (Horner et al. 1988; Nicolai 1988; Palm and Sanchez 1990). Although the stabilization of organic N is mainly associated with condensed tannins (Hagerman and Butler 1991), simple phenolics can also form recalcitrant complexes with amino acids forming humic substances (Martin and Haider 1980) or react with NH_4^+ and NH_3 when phenolics are oxidized to quinones forming polymers where N would be stabilized to microbial attack (Nommik and Vahtras 1982; Stevenson 1982).

7.2.2 N Mineralization

Phenolic compounds have been associated to low net N mineralization – the balance between production and consumption of inorganic N – in litter and organic and mineral soil (Fierer et al. 2001; Fox et al. 1990; Northup et al. 1995; Palm and Sanchez 1990, 1991; Schimel et al. 1996). Since decreases in net N mineralization can be caused either by decreases in gross N mineralization (NH_4^+ production) when availability of organic N for microbes is reduced, or by increases in inorganic N immobilization as a result of higher microbial activity, radio labeled techniques such as ^{15}N isotope dilution have been used to determine the specific mechanisms affected (Hart et al. 1994). Fierer et al. (2001) found a reduced gross N mineralization in organic soil amended with condensed tannins from *Alnus tenuifolia* and *P. balsamifera,* probably as a result of binding organic matter. A similar result was found by Castells et al. (2004) with a decrease in gross N mineralization when soils were amended with phenolics from *C. albidus*.

7.2.3 N Immobilization

Decreases in net N mineralization can also be a result of increases in N immobilization after enhanced microbial activity. Phenolic acids have been shown to stimulate microbial respiration and growth when microbes use them as a C source (Blum and Shafer 1988; Shafer and Blum 1991; Schimel et al. 1996; Souto et al. 2000; Sparling et al. 1981; Sugai and Schimel 1993). In some cases microbial degradation occurs very rapidly. Sugai and Schimel (1993) estimated that 90% of p-hydroxy benzoic acid and salicyclic acid released to mineral soil were metabolized within 4 h. More complex phenolics can also be degraded by soil microorganisms. Positive correlations have been found between N immobilization in litter and condensed tannins concentrations in several Mediterranean species (Gallardo and Merino 1992) as well as increases in NH_4^+ consumption in organic soils amended with condensed tannins of *K. angustifolia* and *Abies balsamia* (Bradley et al. 2000) and *P. balsamifera* (Fierer et al. 2001). Even though all type of phenolics are used as a C source by soil microorganisms low molecular weigh phenolics tend to be degraded more easily by microorganisms than high molecular weight condensed tannins (Fierer et al. 2001). As an example, some species of microorganisms, including filamentous fungi, yeasts and bacteria, with the ability to use hydrolysable and condensed tannins as their sole C source were also able to grow on simple phenolics, but the reverse was not always true (Scalbert 1991). Thus, the importance of simple phenolics on increasing N immobilization in natural conditions seems, at least in short-term, more relevant for the soil N availability than the effect of tannins.

7.2.4 Nitrification

The inhibition of nitrification is probably the more controversial effect of phenolics on N cycling. Rice and Pancholy (1973) and Rice (1974) proposed that phenolic compounds would inhibit growth of nitrifying bacteria, which would explain decreases of nitrification observed along succession in forests soils. Even though studies in vitro have found decreases in the transformation of ammonium to nitrite, and nitrite to nitrate caused by phenolic acids (Hartley and Whitehead 1985) and condensed tannins (Baldwin et al. 1983), those effects have not proved universal (McCarty et al. 1991; Souto et al. 2000). An alternative explanation for decreases in nitrification in the presence of phenolics is related to competition between nitrifying bacteria and heterotrophic microorganisms for the same substrate (NH_4^+). When phenolics are degraded by microbes and immobilization is enhanced, a decrease of nitrification can occur due to a lower NH_4^+ supply but not necessarily by inhibition of the nitrifying bacteria (Castells et al. 2004; Stienstra et al. 1994). The dominance of N immobilization is expected to occur when a limited NH_4^+ supply raises competition between microorganisms (Riha et al. 1986), and in this case, nitrification may appear inhibited when this effect may be only caused by a decrease of the substrate for nitrifying bacteria. Future studies on the effects of phenolics on nitrification will need to determine whether decreases in nitrification are either caused by toxic effects of phenolics on nitrifying bacteria or by changes in substrate availability.

7.2.5 Mycorrhizal Fungal Activity and N2 Fixation

The major role of mycorrhizae is to mobilize nutrients, mainly N and P, present in the soil but unavailable for plant uptake (Read 1991). Inhibition of mycorrhizae can lead, therefore, to a loss of competitive ability for the plant. The response of mycorrhizae to secondary compounds is highly variable, including inhibition or enhancement of fungal activity; this variability seems to depend on the specific chemical structure, concentration and type of fungi (Souto et al. 2000). Several phenolic acids and acid tannic naturally present in the humus were found to inhibit mycorrhizal growth and respiration (Boufalis and Pellissier 1994; Bending and Read 1996).

Foliar leachates from *Rubus idaeus* inhibited growth of five species of ectomycorrhizae associated to *Picea mariana* but no effects were found for other two species (Coté and Thibault 1988). A decrease in oxygen consumption caused by phenolic acids was found in two mycrorrhizal fungi at concentrations of 10^{-7} M, but at higher concentrations (10^{-3} M) respiration was increased (Boufalis and Pellissier 1994).

The effects of phenolics on N2 fixation have been rarely evaluated. Schimel et al. (1998) determined that condensed tannins from *P. balsamifera* inhibited N_2 fixation in *A. tenuifolia* nodules and thus N input into the ecosystem.

7.3 Factors Affecting the Action of Phenolics in Natural Conditions

Experimental data strongly suggests that phenolics can decrease soil N availability in controlled conditions. However, the relevance of phenolics in determining soil N transformations in natural systems depends on the quantity and quality of the phenolics released to the soil, the soil physical and chemical properties, and the relative importance of phenolics in relation to other C compounds released from the plant which can also affect N cycling.

7.3.1 Release of Phenolics

Phenolics are a group of secondary metabolites widely distributed in plants, but their chemical pattern varies extremely within and between species (Waterman and Mole 1994). While the presence of phenolic contents in green foliage has been extensively analyzed, literature is scarce in studies on type and amount of phenolics naturally leached from plants. Gallet and Pellissier (1997) and Gallet (1994) analyzed total phenolics presents in throughfall, snow and soil solutions from a coniferous forest. Significant amounts of phenolic concentrations were found in throughfall collected under *Picea abies* (22–28 mg L^{-1} of gallic acid equiv.) and in the snow melt (1–3 mg L^{-1} of gallic acid equiv.). More commonly, studies on plant–soil interactions have used leachates obtained experimentally, even though concentrations are probably overestimated. A proportion of 20–25 g of foliage per litter of water is commonly used when preparing leachates, which is roughly the volume of snow accumulated per weigh foliage of *E. hermaphroditum* (Zackrisson and Nilsson 1992). Regarding the type of phenolics present in leachates, phenolic acids have been widely reported, specially feluric, coumaric and cinamic acids (Kuiters and Sarink 1986; Singh et al. 1989; Wallstedt et al. 1997; Zhu and Mallik 1994) together with flavonoids (Chaves and Escudero 1997) and condensed tannins (Castells et al. 2004; Gallet and Pellissier 1997). A better knowledge of variability of phenolic compound concentrations released in natural solutions, including inter-specific variability as well as spatial and temporal changes, is necessary to determine the implications of phenolics in plant–soil interactions.

7.3.2 Soil Properties

Soil chemical and physical properties, such as pH, clay content and nutrient status, can play an important role in the fate of phenolic compounds including their activity, retention in the soil system and degradation. Phenolic compounds require oxidation for most of their ecological activities, and their oxidation state varies with the physicochemical conditions of the environment such as soil redox potential (pE and pH) (Appel 1993). Soil pH determines the type and stability of bonds between phenolic compounds and organic matter, which likely affects their lability. At pH > 8 phenolic compounds can form irreversible covalent bonds with organic matter, while at lower pH they tend to form hydrogen bonds which are characterized by their reversibility and lower strength (Appel 1993). Presence of Ca^{++} can reduce the reactivity of phenolic compound functional groups by mediating the formation of bonds between clays and organic matter (Oades 1988). Biological degradation rates of phenolic compounds are also influenced by pH and soil texture. The activity of polyphenol oxidases (PPO), a family of enzymes synthesized by ectomycorrhizal fungi that mediate the first stage of phenolic compound mineralization, has been shown to increase with soil pH (Bending and Read 1995; Leake and Read 1990; Pind et al. 1994) and decrease with high clay content and cation-exchange capacity (Claus and Filip 1990). Thus, under conditions of high pH, carbonate content and clay content, phenolic compounds tend to bind organic N compounds, becoming less reactive and less degradable (Appel 1993; Claus and Filip 1990; Oades 1988).

One example of the importance of soil properties on the fate and effects of phenolics is reported in Castells and Peñuelas (2003). In this study the interaction of the Mediterranean shrub *C. albidus* with N cycling was studied for plants growing on siliceous or calcareous soils within 600 m of each other and under similar conditions of precipitation and temperature. Siliceous soils (granite- and schist-derived soils) had lower pH, electric conductivity, carbonate content, organic matter, organic N and organic P, and higher sand and lower clay content compared to calcareous soil (Castells and Peñuelas 2003). The role of phenolics was significantly different for each bedrock type. Plants growing in calcareous soils had lower foliar phenolic concentrations but the presence of reversibly bound phenolics in the soil was higher compared to siliceous soils, indicating that their degradation was decreased probably as a result of stabilization by polyvalent cations and clay particles. Physical and chemical properties of calcareous soils also determine a lower effect of plant phenolic compounds on N cycling compared to siliceous soils. In calcareous soils, the high content of clays and calcium slowed N cycling rates and resulted in an accumulation of organic matter. The inputs of organic compounds from the plant, including phenolics, represented a low proportion of the total organic matter present in the soil. Thus, the release of C compounds from the plant had a weak effect on N cycling compared to the soil physical and chemical properties, and N cycling under plant was similar to the bare soil. In siliceous soils, however, the absence of major amounts of Ca and clays determined a lower organic matter retention and faster N cycling. In this case, N-cycling was affected by the plant canopy, and phenolics decreased N mineralization as a result of being used as a C source by microorganisms.

7.3.3 Importance of Phenolics in Relation to Other C Compounds

Phenolic compounds are normally released from foliage and decomposing litter together with other soluble C based metabolites such as carbohydrates (Horner et al. 1988). In some cases phenolics were found to be a significant fraction of the DOC (Dissolved Organic Carbon) leached from vegetation. Michelsen et al. (1995) found 232, 200 and 452 mg L^{-1} of total phenolics in leachates of *Cassiope tetragona*, *E. hermaphroditum* and *Betula tortuosa*, respectively, but only 14.5, 20.9 and 28.2 mg L^{-1} of labile carbohydrates. In other cases, however, proportions of phenolics and carbohydrates were lower. In *C. albidus* leachates only the 46% of DOC were phenolic compounds (Castells et al. 2004). Quantifying the relative importance of phenolics from the whole carbon fraction is a pending issue in the study of phenolics in natural systems. Carbohydrates have been shown to increase soil N immobilization when microbes use them as a C source (Blum and Shafer 1988; Castells et al. 2004; Sparling et al. 1981; Sugai and Schimel 1993) and they should also be considered because in some cases the effects of carbohydrates may eclipse the changes produced by phenolics. Castells et al. (2004) quantified the relative importance of the phenolic compounds compared to other soluble C compounds present in the leachates on N cycling. Phenolics were not quantitatively relevant under *Cistus* because the addition of phenolics decreased gross N mineralization, probably by forming bonds with proteins, while soils sampled under the canopy had higher gross N mineralization rates compared with control soils. These results suggest that, although the decrease in gross N mineralization caused by phenolic compounds potentially occurred under natural conditions, the release of other compounds from the plant, including aboveground inputs of labile C compounds from leaves and litter and below-ground inputs from root exudation or root decomposition, were likely to have stronger effects on N mineralization.

7.4 Evidences of Decrease in N Availability Under Natural Conditions

The effects of phenolics on N cycling have been mostly tested by adding to the soil a single phenolic or a mixture of phenolic compounds either synthetics or purified from plant tissue (Blum 1998; Blum and Shafer 1988; Boufalis and Pellissier 1994; Bradley et al. 2000; Fierer et al. 2001; Inderjit and Mallik 1997; Magill and Aber 2000; Schimel et al. 1996; Shafer and Blum 1991; Sparling et al. 1981; Sugai and Schimel 1993). However, several problems arise when using this approach to determine the role of phenolics under natural conditions. First, phenolic compounds leached from the plant foliage and leaf litter span over a range of molecular weights and have different abilities to interfere with N cycling (Fierer et al. 2001; Hättenschwiler and Vitousek 2000) and consequently the effects of a single phenolic compound or a partial mixture may not account for the overall effects of the phenolics released to the soil (Inderjit and Mallik 1997). Another problem derived from those experimental designs is that normally only one source of phenolics is evaluated, either leachates from green foliage, decomposing litter or root exudates, underestimating the amounts of phenolics released to the soil. Moreover, the importance of phenolics compared to other compounds as well as their fate depending on the soil physical and chemical properties, as described above, must be considered. In order to ascertain the relevance of phenolics in N cycling under natural conditions and possible consequences on plant communities a more global approach needs to be followed, for instance determining the changes in N availability associated to the presence of a phenolic-producer.

In general, soils underneath a plant have more organic matter and soil microbial activity (Aguilera et al. 1999; Hook et al. 1991; Vinton and Burke 1995) and significantly higher nutrient availability (Facelli and Pickett 1991; Vinton and Burke 1995) compared to bare ground. However, if phenolic compounds released to the soil control N cycling dynamics underneath the canopy we would expect to find lower N availability under a phenolic producer compared to areas where this species is not present. In the following section I review five examples in which the effect of plant canopy from one Mediterranean and four boreal species decreased inorganic N production in natural conditions. To my knowledge these studies were the only work in literature that met the following criteria: (1) the species involved contained high phenolic concentrations, (2) the phenolic-producers were associated with decrease in growth of other plants growing nearby, (3) the experiments were designed to test the effects of allelochemicals in natural conditions, and (4) laboratory studies conducted with leachates or purified phenolics were available to complement the results obtained in the field.

7.4.1 N Transformations Under *Cistus albidus*

Cistus albidus is a Mediterranean evergreen pioneer shrub that tends to dominate shrubland ecosystems after recurring fires, forming in some cases dense and almost monospecific stands (De Luis et al. 2006; Juhren 1966). The post-fire dominance of Cistaceae species, including *C. albidus* and *C. ladanifer*, has been partially attributed to the release of carbon-based secondary metabolites to the environment (Chaves et al. 2001; Robles et al. 1999). *C. albidus* releases high concentrations of phenolic compounds from green leaves and decomposing litter compared with other Mediterranean shrubs from the same plant community (Castells et al. 2004). Castells and Peñuelas (2003) and Castells et al. (2004) analyzed N transformations under *C. albidus* individuals and in a nearby control site free from *C. albidus* in three bedrock types. Soils under *C. albidus* had lower net N mineralization, higher C mineralization and lower ratio of N-to-C mineralized than the control ground. Experiments with ^{15}N indicated that decreases in net N mineralization under canopy were mostly a result of increasing immobilization of NH_4^+ when soluble C released from the canopy were degraded by microbes. The effect of phenolics from green leaf leachates was not quantitatively relevant under *C. albidus* because the addition of phenolics decreased gross N mineralization, while soils sampled under the canopy had higher gross N mineralization rates compared with control soils. Thus, although the decrease in gross N mineralization caused by plant phenolics was potentially occurring under natural conditions, the presence of C compounds from other sources, including labile C compounds and simple phenolics from litter and below-ground inputs, are likely to have stronger effects on net N mineralization and thus on determining N cycling under *C. albidus*.

7.4.2 N Transformations Under *Ledum palustre*

Ledum palustre and *Ledum groenlandicum* are late successional evergreen shrubs widely distributed in boreal ecosystems that are known to inhibit the growth of *P. mariana* and *Picea glauca* when they dominate the understory (Cole et al. 2003; Inderjit and Mallik 1996b). This interference has been related to the presence of plant secondary metabolites (Inderjit and Mallik 1997). *L. palustre* readily leaches high concentrations of phenolic compounds into water (Castells et al. 2004), and amendments of *L. groenlandicum* foliage and litter have been found to increase concentrations of phenolic compounds in the soil (Inderjit and Mallik 1997). Castells et al. (2004) analyzed net N mineralization in mineral soils under *L. palustre* from a hardwood forest in interior Alaska dominated by *Populus tremuloides* and *Betula neoalaskana*, and compared it with nearby control plots where *L. palustre* was not present. Mineral soils sampled under *L. palustre* canopy had a lower net N mineralization and N-to-C mineralization ratio, and a higher C mineralization compared to control soils, which was caused by higher gross ammonium immobilization rates (110.6% increase) compared to *Ledum*-free sites. The canopy effects could be partially caused by the compounds leached from foliage because soils sampled under *L. palustre* and amended with leachate showed similar responses. Thus, C compounds leached from *L. palustre* stimulated microbial activity when microbes use them as a substrate resulting in increases in soil N immobilization and decreases in N availability for vegetation.

7.4.3 N Transformations in the Presence of *Kalmia angustifolia*

Regeneration failure of conifers in boreal forests dominated by understory ericaceous species (*K. angustifolia*, *E. hermaphroditum*, *Vaccinium myrtillus* or *Gaultheria shallon*) has been partially explained by allelopathy (Inderjit and Mallik 2002) and partially by the indirect effects of phenolics on changing soil fertility (Mallik 2003; Ponge et al. 1998). Particularly, the effects of *K. angustifolia* on inhibiting conifer growth have been extensively studied, mostly from the allelopathic point of view (Inderjit and Mallik 1999; Inderjit and Mallik 2002; Mallik 1995). Fresh leaves of *K. angustifolia* contain ferulic, vanillic, syringic, gentisic, m-coumaric, p-coumaric, o-hydroxyphenylacetic, and p-hydroxybenzoic acids and these where found to inhibit root growth of black spruce (*P. mariana*) (Zhu and Mallik 1994). Changes in N cycling due to *K. angustifolia* phenolics have also been studied by Bradley et al. (1997) incubating soils from a birch site, with absence of *K. angustifolia*, and from sites with low and high presence of *K. angustifolia*, and analyzing soil N process rates. Soils from *Kalmia*-associated sites showed a lower humus chemical quality, with lower extractable NH_4^+ and higher concentrations of tannins and other phenolics compared to soils with no *Kalmia*, as well as an increase of gross N mineralization and N immobilization. The low N availability associated to *K. angustifolia* could be related to higher lignin concentrations or higher concentrations of tannins forming stable complexes with organic N (Bradley et al. 1997). Inderjit and Mallik (2002) also suggested that *K. angustifolia* phenolics could cause a depletion of N by enhancing microbial activity when phenolics were used as C source by microorganisms, although a direct evaluation of these mechanisms has not been conducted. Zeng and Mallik reported that certain ectomycorrhizal fungi associated with black spruce such as *Paccillus involutus* can degrade *Kalmia* phenolics and use the degraded products as their carbon source resulting growth enhancement of black spruce (Zeng and Mallik 2006).

7.4.4 Changes in N Cycling Along a Fire Chronosequence in Boreal Forests

DeLuca et al. (2002) studied the forest soils in a chronosequence spanning 350 years after the occurrence of a wild fire. They analyzed vegetation cover, net N mineralization, nitrification and phenolic concentrations in soils to determine whether changes in soil N transformations along the chronosequence were related to the understory composition and concentrations of phenolics in the forest floor. The presence of ericaceous shrubs, including the dominant *E. hermaphroditum*, increased along time, and this was accompanied by higher phenolic concentrations in soils and lower net N mineralization, ammonification and nitrification. DeLuca et al. (2002) concluded that decreases in net N mineralization were probably a result of increasing rates of N immobilization as phenolics were degraded by soil microorganisms, and thus phenolics were a potential factor determining the availability of inorganic N along succession. Indeed, the boreal shrub *Empetrum hemaphroditum* could have a prominent role in this process because it may become dominant in the understory (Ponge et al. 1998) and it has been reported to contain an array of phenolics, including batatasin-III, which are known to inhibit *P. sylvestris* seedling growth (Nilsson and Zackrisson 1992; Zackrisson et al. 1997). The decreases in net N mineralization when leachates of *E. hermaphroditum* were experimentally added to mineral soil (Castells et al. 2005) are also in agreement with the role of this species on determining changes in soil N cycling.

7.4.5 Changes in N Cycling During Succession from Alder (*Alnus tenuifolia*) to Poplar (*Populus balsamifera*)

The study of succession from alder (*A. tenuifolia*) to poplar (*P. balsamifera*) constitutes one of the best documented cases where phenolics are controlling soil N dynamics and distribution of vegetation (Schimel et al. 1996). In boreal forests, the transition from alder to poplar is accompanied by strong changes in N dynamics. During the alder stage inputs of N are principally due to N_2-fixation, and rates of mineralization and nitrification are increased. However, as poplar gets dominant, N cycling rates are slowed down and organic C accumulation increased (Schimel et al. 1998). Clein and Schimel (1995) investigated the causes of changes in N cycling as poplar replaced alder, and the importance of N cycling changes in the transition between these species, by performing a reciprocal soil transplanting experiment. Soils from alder-dominant sites transplanted to poplar-dominated sites showed a decrease in N availability caused by an enhanced microbial activity and N immobilization when inputs of C compounds were supplied by poplar, while transplanting poplar soils to alder sites had the opposite effect (Schimel et al. 1998). Poplar produces high amounts of condensed tannins and low-molecular weight phenolics (Schimel et al. 1996; Schimel et al. 1998), but although condensed tannins inhibited gross N mineralization in laboratory conditions (Fierer et al. 2001; Schimel et al. 1996) this effect was not occurring in the field (Clein and Schimel 1995). Only labile phenolics were related to changes in N transformations under natural conditions (Clein and Schimel 1995).

The examples above show a decrease in N availability associated to phenolic-compound containing species in natural conditions. In all cases this was attributed to increases in N immobilization when C compounds were used by microbes as an energy source. Changes in N cycling under the canopy may be caused by the release of phenolics into the soil although the effects of other soluble C compounds cannot be excluded. The role of phenolics on increasing N immobilization in natural systems is unexpected because it is normally accepted that the phenolics are mostly involved is the formation of complexes with proteins delaying organic matter decomposition and gross N mineralization. Although this process indeed occurs, a global analysis of the effect of plant canopy on N cycling, as shown in the examples above, excluded it as a main factor driving N cycling under these vegetations. Whether this trend is general or not for other phenolic-containing species and associated soils will have to be further investigated.

7.5 Ecological and Evolutionary Relevance of Phenolics on Decreasing N Availability

The effects of phenolics on plant growth in natural systems have been mostly studied in the context of regeneration of conifers in boreal forests. Some authors have argued that the presence of allelopathic compounds, such as phenolic compounds, leached from ericaceous species in the understory could partially explain the low regeneration of conifers after clearcut disturbance (Ponge et al. 1998). Normally direct and indirect effects of phenolics on conifer growth are considered together, and thus the relative importance of each process has not been quantified. Although indirect effects of phenolics through changing soil N cycling are normally regarded as complementary to allelopathy, they could actually be more important for plant dynamics and ecosystem functioning (Inderjit and Weiner 2001). Depending on the predominant processes in natural ecosystems, the ecological and evolutionary consequences would be different. Since allelopathy requires a species-specific coupled system of secondary metabolites and their targets, this process could be less widespread than changes in soil N availability through fueling soil microbial activity (but it can do so by inhibiting natural regeneration of tree species see Mallik 2003; Wardle et al. 1998). Ecologically, the latter could have a greater potential impact on the ecosystem because changing soil quality may in turn modify performance in many plant species, ultimately affecting plant community structure (Clein and Schimel 1995). Moreover, allelopathy is more likely to occur in low diversity communities where the species producing secondary metabolites could be dominant (Wardle et al. 1998).

Phenolics could also have an important evolutionary role on increasing plant competitive ability against other plant species through changes in the soil nutritional qualities (Horner et al. 1988; Muller et al. 1987; Northup et al. 1998). Phenolic compounds could exert a conservative pressure on soil nutrients by binding proteins and delaying decomposition and mineralization, and thus slowing N losses (Bending and Read 1995; Northup et al. 1995, 1998). Although N availability in the soil would decrease, organic N would be specifically available for those species producing phenolics by the action of mycorrhizae (Read 1991). If this process enhances N conservation in the soil higher phenolic compound concentrations would be selected during evolution particularly in those plants growing on N-limited soils (Bending and Read 1995; Northup et al. 1995, 1998). However, this positive feedback between soil N availability and production of phenolics in foliage has only been found in one case (Northup et al. 1998) while in other species the lower soil N availability caused by phenolics was not positively correlated with an increase of phenolics in foliage (Castells and Peñuelas 2003; but see Bloom and Mallik 2004). Bloom and Mallik (2004) reported from post-fire *Kalmia*-black spruce communities of eastern Canada that reduced N availability of soil is correlated with decreased leaf area and increased phenolic concentrations of *Kalmia* foliage. Even if the feedbacks between N availability and phenolics are not general for all plant–soil systems, the evolutionary importance of phenolics is shown by the presence of mycorrhizae able to degrade phenolic–protein complexes and detoxify simple phenolics coupled with plants containing phenolic compounds (Read 1991; Zeng and Mallik 2006).

7.6 Concluding Remarks

Phenolic compounds can decrease soil N availability creating an unfavorable environment for plant growth. Although many studies have evaluated the effect of phenolics on decomposition, mineralization, immobilization and nitrification in laboratory conditions, few studies have determined the role of phenolics in natural systems. The studies reviewed here show that the main mechanism that changed N cycling under vegetation was an increase of microbial activity when labile phenolics were used as a C-source by microorganisms, as opposed to decreases in gross N mineralization by forming complexes with organic N as traditionally thought. The role of phenolics on outcompeting other species by changes in N cycling could be more relevant than allelopathy in plant distribution dynamics because all plant species might be potential targets. However, additional field studies are needed to better understand the role of phenolics in ecosystem functioning. The field of allelopathy, in particular, and soil chemical ecology, in general, would greatly benefit from distinguishing the specific mechanisms in which secondary metabolites affect plant performance, with special attention to separate the direct effects of phenolics on plant physiology from the indirect effects through changes in soil nutrient availability.

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8. Genomic Approaches to Understanding Allelochemical Effects on Plants

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Abstract. Little is known concerning the mode of action of allelochemicals or plant defense responses mounted against them. Theoretically, changes in the expression of genes encoding the primary target or other proteins in the same pathway should occur soon after phytotoxin exposure. Defense responses, such as the induction of genes involved in chemical detoxification, may occur later, depending on the nature of the chemosensors which presumably exist in plant cells. We first used yeast (*Saccharomyces cerevisiae*) to test the concept of transcriptome profiling of toxicant modes of action. Characteristic gene induction profiles related to specific molecular target sites were verified with several fungicides. A battery of xenobiotic defense-associated genes were found to be dramatically induced in Arabidopsis following exposure to an array of structurally unrelated xenobiotics, including a herbicide, an allelochemical, and herbicide safeners. These genes are unlikely to be strongly linked to the mode of action of a specific phytotoxin, but rather constitute a coordinately-controlled xenobiotic defense gene network. Transcriptional profiling experiments using microarrays are being conducted to examine the effects of various herbicides and natural phytotoxins on the *Arabidopsis* transcriptome.

8.1 Introduction

Advances in molecular biology have provided powerful new tools that can be used to understand complex processes in allelopathy. In this paper, we provide examples of how whole genome transcriptome analyses with DNA microarrays might be used to provide clues about the mode of action of allelochemicals, as well as the mechanisms of defense against allelochemicals and the biosynthesis of allelochemicals. We will summarize our research in this area and discuss it in the context of relevant research. This chapter is an updated revision of a previously published review (Duke et al. 2005b).

8.2 Transcriptome Profiling of the Mode of Action of Allelochemicals

Detection of the global expression response of plant genomes after treatment with phytotoxins is possible with DNA microarrays. Theoretically, at a given dose of a phytotoxin, at a specific time after exposure to the phytotoxin, one might expect changes in the transcriptome that would be specific for phytotoxins with the same molecular target site (Eckes et al. 2004; Duke et al. 2005a). Thus, one can generate a library of transcriptome profiles for phytotoxins with different molecular target sites that would be useful in the determination of the molecular targets of phytotoxins with unknown sites of action. This approach has been used in pharmaceutical research.

For example, Boshoff et al. (2004) generated 430 transcription microarray profiles of inhibitors of *Mycobacterium tuberculosis*. The profiles of compounds with known modes of action were useful in determination of several compounds with unknown modes of action. Analysis of these data revealed 150 clusters of coordinately regulated genes, and a signature subset of these gene clusters was sufficient to classify all known agents as to mechanism of action of anti-tuberculosis drugs. Transcriptional profiles generated by a crude marine natural product generated the same prediction of a mode of action as the pure active component.

There are several potential problems with this approach. Any toxicant that kills an organism will potentially affect a huge number of genes at some doses and times after exposure. Consequently, determining the proper dose and time after treatment for best seeing effects on transcription of genes directly related to the molecular target site is important, and not a simple endeavor. Toxicants also induce genes associated with stress and protection from xenobiotics. Sometimes these effects can be very dramatic compared to effects on genes associated with the molecular target site. Many target sites are associated with genes that are normally well expressed, encoding gene products necessary for fundamental cellular functions. Thus, there may not much latitude for dramatic changes in expression of these genes. Sifting through the huge amount of data generated from microarray experiments to find effects on genes related to the target site of the toxicant can be challenging.

We began our studies of mode of action with microarrays by examining the effects of agricultural fungicides on yeast (*S. cerevisiae*), using whole genome cDNA chips (Kagan et al. 2005). There are several advantages of *S. cerevisiae* over plants for this type of study. First, there is only one cell type, so effects of a toxin on tissue- or cell-type specific genes are not diluted by lack of effect on these genes in other tissues or cell types. Second, all cells can be treated rapidly and uniformly with the toxicant, unlike the situation with whole plants. Third, the number of genes in the yeast genome is significantly smaller than in that of *Arabidopsis thaliana*, a plant with a very small genome compared to most other plants. Lastly, the functions of yeast genes are better annotated in *S. cerevisiae* than those of any higher plant, making it more likely that effects of toxins on this organism's transcriptome can be more readily understood.

Our strategy has been to find reproducible effects on specific genes or groups of genes that can be linked to a molecular target site. We tested the effects of eight fungicide inhibitors of ergosterol synthesis, representing three classes of these inhibitors targeting three different target sites of the pathway (Fig. 1). A putative inhibitor of methionine synthesis, cyprodinil, was also examined. Characteristic changes in gene transcription for the genes of the ergosterol pathway were seen for Class I and Class II inhibitors.

This pattern was not found for the Class III ergosterol inhibitor, which would be expected to affect ERG27 gene expression, nor was it found with cyprodinil. Cyprodinil caused upregulation of three genes involved in methionine metabolism, and there were essentially no effects of the other ergosterol inhibitors on methionine synthesis genes. From these results, the effect of the Class III ergosterol inhibitor on the ergosterol synthesis pathway in *S. cerevisiae* is questionable. Using oligonucleotide microarrays, we have generated unpublished results that link other fungicides with known modes of action to genes related to their target sites. Although we still have much work to do with yeast, the concept of transcriptome profiling appears to be valid for fungicides that inhibit growth of yeast.

Similarly, Gutteridge et al. (2005), working with *S. cerevisiae*, in a search of the mode of action of a potential agricultural fungicide, found that with some compounds specific gene clusters were affected in ways that provided clues to their mode of action. Agarwal et al. (2003) found that *S. cerevisiae* responded to pharmaceutical fungicides with several different molecular target sites with drug-specific effects on gene transcription.

Yeast is not a good model for herbicide mode of action or for many plant processes (Bassham and Raikhel 2000), so plant microarrays must be used to probe the mode of action of herbicides and phytotoxins. Companies involved in herbicide discovery apparently have extensive plant transcriptome profile libraries for herbicides with different modes of action, although no details of their results have been published. A very few publications exist on transcriptome profiles for individual phytotoxins (2,4-D, Raghavan et al. 2005a,b; bromoxynil and two sufonylurea herbicides, Glombitza et al. 2004; isoxaben, Manfield et al. 2004; thaxtomin A, Scheible et al. 2003; coronatine, Uppalapati et al. 2005; and flufenacet, Lechelt-Kunze et al. 2003). There is one report of DNA microarray methods leading to the discovery of the mode of action of an allelochemical. Bais et al. (2003) reported that (–)-catechin is phytotoxic to Arabidopsis due to an effect on a calcium ion signaling cascade. This result has not yet been confirmed by other laboratories.

Our laboratory has initiated work on establishing a database of phytotoxin-related transcriptome profiles. Our first experiment in this endeavor was to examine the effect of the allelochemical benzoxazolin-2(3H)-one (BOA) on gene expression in Arabidopsis. Careful dose response experiments (Fig. 2) allowed us to determine I_{50} and I_{80} concentrations for root growth inhibition. Then, plants were grown for 10 days and exposed to these concentrations of BOA, after which mRNA was extracted and analyzed with Affymetrix *Arabidopsis* ATH1 Genome Arrays (Baerson et al. 2005).

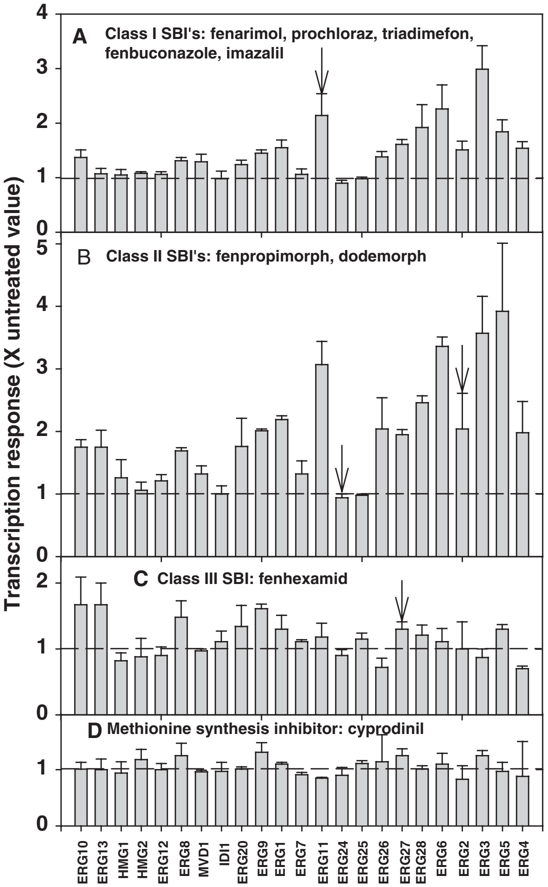

Fig. 1. Effects of Class I (**A**), II (**B**) and III (**C**) ergosterol biosynthesis inhibitors, and a putative methionine biosynthesis inhibitor (**D**) applied at the I_{50} concentrations for 2 h on expression levels of genes in the ergosterol pathway. Standard errors are shown in A and B, and standard deviations are shown in C and D. Genes are listed on the *x*-axis from left to right in the order in which they appear in the pathway. The transcription relative to untreated controls is shown on the *y*-axis. Dashed horizontal lines on the graphs indicate the level of expression at which no change is seen relative to the control. Arrows indicate gene(s) targeted by the inhibitor (from Kagan et al. 2005)

One hundred-fifty-eight genes were significantly induced, and 30 were repressed in both the I_{50} and I_{80} treatments, totaling approximately 0.8% of all genes represented on the ATH1 gene chip. The breakdown of categories of genes that were affected is provided in Fig. 3. Unfortunately, the exact mode of action of BOA is unknown, so we did not know what genes to focus on regarding the mode of action. We will need a more complete library of transcriptome responses to phytotoxins with different modes of action in order to better interpret the results of this experiment. Meanwhile, physiological approaches continue to provide information that might help us understand the mode of action of benzoxazolinones (e.g., Kato-Noguchi and Macias 2006). Nevertheless, our results with BOA revealed a considerable amount of information about the responses of *Arabidopsis* to an allelochemical, in terms of how the plant protects itself from such a chemical threat.

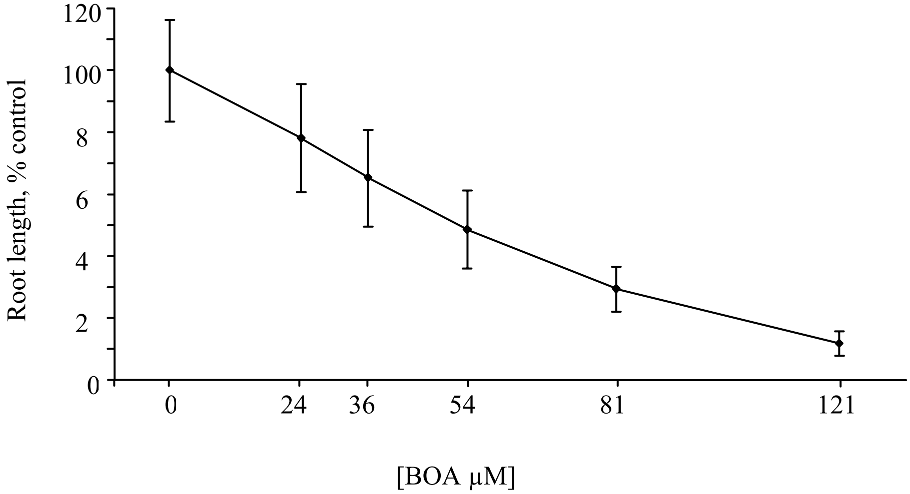

Fig. 2. Dose-response experiments on effects of BOA on root length of Arabidopsis. Each data point represents mean root length from two independent replicates ± 1 SD (adapted from Baerson et al. 2005)

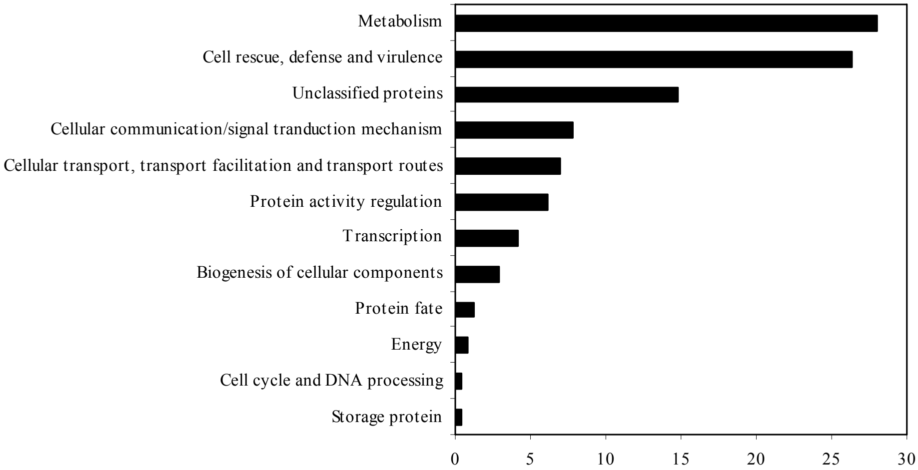

Fig. 3. Distribution of BOA-induced genes into functional categories

8.3 Using Transcription Responses to Understand Plant/Plant Interactions

8.3.1 Defenses Against Allelochemicals

Non-phytotoxic compounds can also induce genes that provide defenses against phytotoxins. This is the principle of crop safeners that are used to protect crops from herbicide injury. Microarray technology has been used to probe the mechanism of action of safeners. Using a cDNA microarray, genes of hybrid poplar (*Populus nigra* × *Populus maximowiczii*), Rishi et al. (2004) found differentially transcribed genes in response to a safener. Genes encoding enzymes involved in oxidation, conjugation, and sequestration of xenobiotics were found to be upregulated by the safener. Little has been done with molecular biology to determine how plants protect themselves from allelochemicals. Matvienko et al. (2004) found genes encoding quinone oxidoreductases to be upregulated by treatment of plants with allelopathic 2,6-dimethoxybenzoquinone, indicating that this enzyme is involved in detoxification of the compound. In earlier work examining the upregulated genes in response to this quinone, several genes encoding enzymes predicted to detoxify the quinone were found to be upregulated (Matvienko et al. 2001).

Our work with BOA effects on the Arabidopsis transcriptome was even more informative (Baerson et al. 2005). As shown in Fig. 3, genes encoding proteins related to cellular defense were the second largest category of genes induced by BOA. Table 1 lists some of the most affected genes in this category.

The effect of BOA on transcription of these genes was more quantitatively determined with quantitative real time RT-PCR (Fig. 4). In most cases, the level of induction was similar, however, in a few cases the microarray method underestimated the level of upregulation. We were amazed that the massive detoxification response of this plant to this allelochemical. This led us to try to determine whether any of the metabolic detoxification products known to occur in plants (Fig. 5) were present.

We found three of these metabolites in BOA-treated Arabidopsis (Table 2), indicating that at least three detoxification enzymes that were induced helped to detoxify this phytotoxin. The primary metabolites were BOA-6-OH and its glucoside, which most likely requires a cytochrome P450 and a UDP glucosyltransferase to be produced. Several representatives of each of these enzymes were upregulated in response to BOA exposure. Further experiments, using quantitative real time RT-PCR, revealed that most of the genes of Table 1 and Fig. 4 are induced by a wide range of xenobiotics, including 2,4-D, two herbicide safeners, and phenobarbitol (Baerson et al. 2005). Our results suggest that allelochemicals induce a wide range of genes involved in detoxification of potential phytotoxins.

Table 1. Selected genes of *Arabidopsis* associated with detoxification of xenobiotics that were most highly induced by BOA as measured by microarray analysis (adapted from Baerson et al. 2005)

Locus ID	Gene description	Fold increase	
		I_{50}	I_{80}
At1g15520	ABC transporter	2.1	10.8
At3g28740	cytochrome P450 family	3.9	16.9
At3g04000	short-chain type dehydrogenase/reductase-related	5.1	15.0
At1g17170	glutathione transferase, putative	6.9	18.1
At5g13750	MFS antiporter	2.5	3.9
At5g16980	quinone oxidoreductase, putative	3.2	9.4
At4g20860	FAD-linked oxidoreductase family	8.4	9.3
At2g19190	light repressible receptor protein kinase, putative	8.4	7.6
At5g27420	ING-H2 zinc finger protein-related	4.6	6.6
At4g34135	glucosyltransferase-related protein	9.4	18.3
At1g05560	UDP-glucose transferase (UGT1)	6.4	14.6
At2g15480	glucosyltransferase-related protein	5.2	12.0
At2g15490	glucosyltransferase-related protein	3.3	10.4
At5g39050	malonyl transferase	3.0	5.5
At4g12490	PEARL1 related	30.1	26.6
At4g12500	PEARL1 related	16.5	10.5

8.3.2 Elicitation of Allelochemical Defenses

Use of whole-genome microarrays should be useful in the future for elucidating the genetics and enzymology of allelochemical biosynthesis. Kong et al. (2004) recently found that weeds can induce biosynthesis of allochemicals in allelopathic rice, in much the same way that pathogens induce phytoalexin production by plants. One of the genes for an enzyme involved in biosynthesis of momilactone B, a rice allelochemical and phytoalexin (Kato-Noguchi and Ino 2005), was induced in rice leaves (Xu et al. 2004). The whole-genome rice microarray was not used in this study. Since high quality, whole-genome microarrays are available for rice, this technology could be very useful in identifying the entire biochemical pathway for all of the induced allelochemicals of rice. Furthermore, this approach could provide valuable information on how these pathways are regulated.

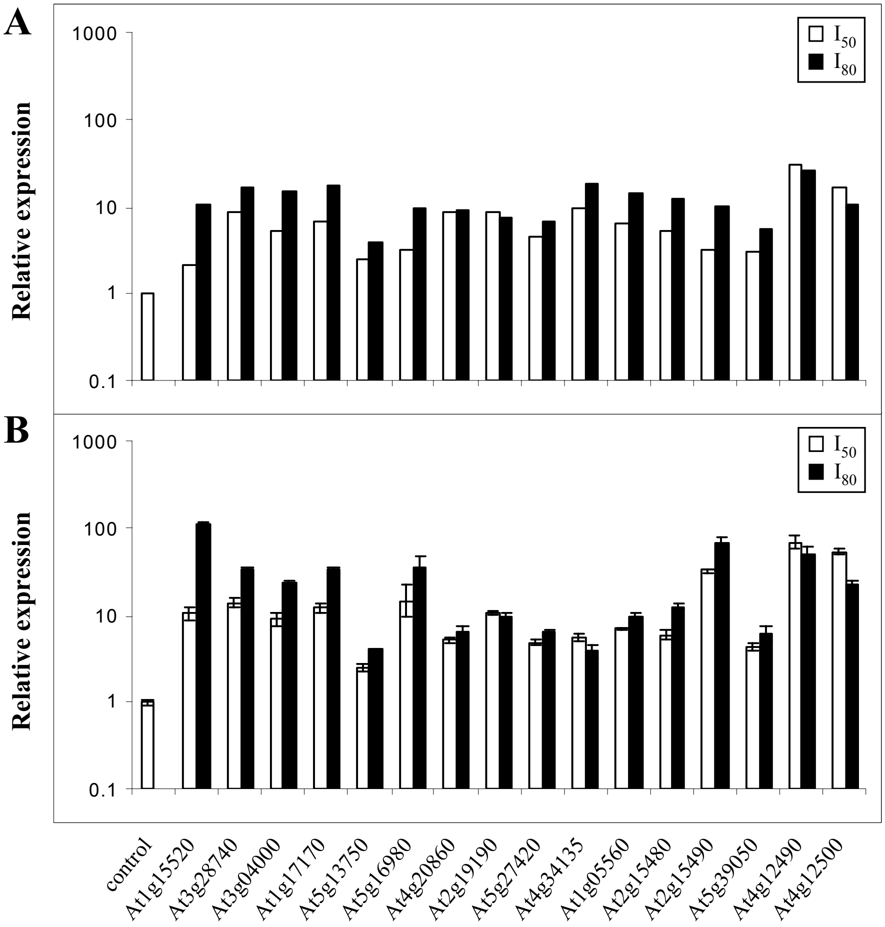

Fig. 4. Sixteen representative genes identified as differentially expressed by microarray analysis (Table 1) were analyzed by quantitative real-time RT-PCR using gene-specific primer pairs. (**A**) Transcriptional profiling results. Shown are relative gene expression values obtained from microarray experiments with BOA-treated Arabidopsis seedlings. The data represent selected genes up-regulated in both I_{50} (open bars) and I_{80} (closed bars) BOA treatments. (**B**) Quantitative real-time RT-PCR results. The RNA samples used were identical to those used for transcriptional profiling results shown in panel A. Data were normalized to an internal 18S ribosomal RNA control. Data are means ± 1 SD (adapted from Baerson et al. 2005)

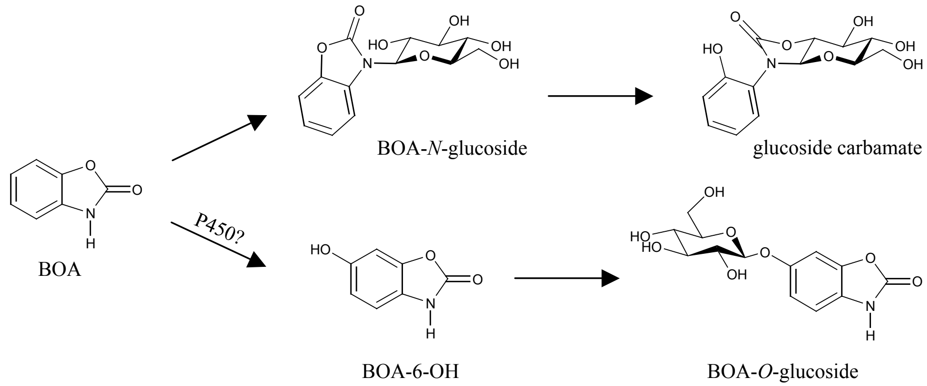

Fig. 5. Two metabolic detoxification of BOA schemes known to occur in plants

Table 2. Metabolites of BOA were quantified by HPLC for three-week-old plants exposed to 10, 100, 250, and 500 µM concentrations of BOA for a period of 24 h. A minimum of 30 plants were used per treatment. Each datum represents the mean from three replicates ± S.D. (adapted from Baerson et al. 2005)

	Metabolite (nmol/g fresh weight)		
[BOA]	BOA-6-OH	BOA-6-*O*-glucoside	Glucoside carbamate
10 µM	20.3 ± 4.0	23.8 ± 5.3	n.d.
100 µM	66.0 ± 10.0	121.5 ± 41.8	33.5 ± 32.8
250 µM	174.8 ± 58.1	249.3 ± 90.0	38.7 ± 20.0
500 µM	212.3 ± 35.6	381.3 ± 147.7	54.8 ± 10.0

Summary

Allelopathy is one of the last areas of plant science to employ molecular biology as a tool in understanding the phenomenon. We and others have provided a tiny glimpse of what might be done with the powerful technique of transcriptional profiling using whole genome microarrays. In the future, we hope to generate a much more complete transcriptional profile for phytotoxins with different modes of action. As such a database becomes more complete its use in providing clues to the modes of action of allelochemicals will become more robust. We also intend to use this technology to examine biosynthesis of allelochemicals and to further study plant defenses against allelochemicals.

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9. Allelopathy from a Mathematical Modeling Perspective

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Abstract. Of the disciplines involved in allelopathy research, mathematical modelling is making increasingly significant contributions. This chapter discusses, from a point of mathematical modelling view, some fundamental issues in allelopathy research, such as hormesis phenomenon and its interpretation, function of allelopathy and its relationship with competition, and periodic production of allelochemicals and stress.

9.1 Introduction

Allelopathy is the release of chemicals by one plant that affect other plants in its vicinity, usually to their detriment. Molisch (1937) coined the term allelopathy to include both harmful and beneficial biochemical interactions between all types of plants, including micro-organisms. Rice (1984) reinforced this definition in the first monograph on allelopathy. Contemporary researchers have broadened the context of allelopathy to include interactions between plants and higher animals, and have suggested that allelopathy may be part of a whole network of chemical communication between plants, and between plants and other organisms, including bacteria, yeasts, insects and mammals; such communication may contribute to plant defence (Harborne 1987; Lovett and Ryuntyu 1992; Einhellig 1995; Siemens et al. 2002). During the last two decades, the science of allelopathy has attracted a great number of scientists from diverse fields worldwide and is now viewed with a multifaceted approach (Rice 1984, 1985; Putnam and Tang 1986; Rizvi and Rizvi 1992; Inderjit et al. 1995, 1999; Narwal et al. 1998; Macias et al. 1999; Chou et al. 1999; Kohli et al. 2001; Harper et al. 2005). This has been greatly driven by the prospect that allelopathy holds for meeting increased demands for sustainability quality in food production for humans, for reducing environmental damage and health hazards from chemical inputs, for minimizing soil erosion, and reducing our reliance on synthetic herbicides, by finding alternatives (Einhellig 1995; Dakshini et al. 1999; Singh et al. 2001; Harper et al. 2005).

Of the disciplines involved in allelopathy research, mathematical modelling efforts have been increasing. Such theoretical contributions range from separating allelopathy from competition (Weidenhamer et al. 1989; Nakamaru and Iwasa 2000; Liu et al. 2005), characterizing allelopathy and its ecological roles (Cheng 1995;

Dubey and Hussain 2000; Goslee et al. 2001), elucidating fundamentals of allelopathy (An et al. 1993; Mukhopadhyay et al. 1998; Liu et al. 2003), simulating specific cases, i.e., plant residue allelopathy (An et al. 1996; Sinkkonen 2003) and algae allelopathy (Sole et al. 2005), to modelling effects by external factors such as density of target plants (Weidenhamer et al. 1989; Sinkkonen 2001, 2005), to applying various mathematical models for better analysis of allelopathy data (An et al. 2005; Liu and An 2005) and further improve the existing allelopathy models (Martins 2006). These theoretical mathematical models, together with other disciplines, have contributed to increasing our understanding of allelopathy, establishing the fundamentals of allelopathy and function of allelochemicals, highlighting directions for future research by integrating scattered information, generalising the phenomena observed in fields and laboratories and providing theoretical framework and insights into the mechanism of allelopathy phenomena.

This chapter, mainly based on our previous modelling work, discusses some fundamental issues in the allelopathic phenomena from the perspective of mathematical modelling and to further illustrate the above mentioned contributions that mathematical modelling can make to allelopathy research.

9.2 Allelopathy and Chinese Yin/Yang Theory

Up until now, one of the most consistent features in studies of allelopathy has been the recognition of the characteristic responses of an organism to an allelochemical, i.e., the stimulation or attraction at low concentrations of allelochemicals and inhibition or repellence as the concentration increases (Lovett 1979; Rice 1984; Belz and Hurle 2002; Liu et al. 2003). These phenomena have also been widely recognized in other growth-regulating chemicals, including herbicides (Devlin and Witham 1983; Fedtke 1982; Calabrese and Baldwin 2003). However, we are still facing the challenge of interpreting such a phenomena and its significance is yet to be fully explored.

Yin/Yang theory is a philosophy of ancient China and recognizes that the ancients understood the character of nature and its laws of development and change. The theory is now widely applied in modern sciences, such as astronomy, physics, mathematic and biology. This theory proposes that all forces occur in complementary pairs, Yin and Yang, which represent two primary cosmic principles. Yin/Yang act in a way that is antagonistic to each other, as well as coexistent. As a unity of opposites, Yin and Yang, although separate forces, are really part of the same ultimate unity, i.e., Yin implies Yang, Yang embodies Yin, and each is, therefore, necessary to the other. It is through this interaction of these two primal forces that all of the phenomena of the universe are produced (Fig. 1).

Being inspired by this theory, an analogy is employed to interpret allelopathic manifestations. It is hypothesized that the characteristic response to allelochemicals is a result of the character of the allelochemicals themselves. An allelochemical is assumed to have two complementary attributes: stimulation and inhibition. These attributes act in a way that is antagonistic to one another as well as coexistent within the unity of an allelochemical. As a unity of opposites, stimulation and inhibition, although separate attributes, are really part of the same ultimate unity, i.e., stimulation implies inhibition, inhibition embodies stimulation, and each is, therefore, necessary to the other. As concentration changes, the relative dominance of stimulation and inhibition of the allelochemical over each other is altered. It is this alternation that determines the overall property of the allelochemical. This can only be shown through the biological responses when an allelochemical acts on an organism, and is referred to as the biological property of the allelochemical (as opposed to chemical or physical properties).

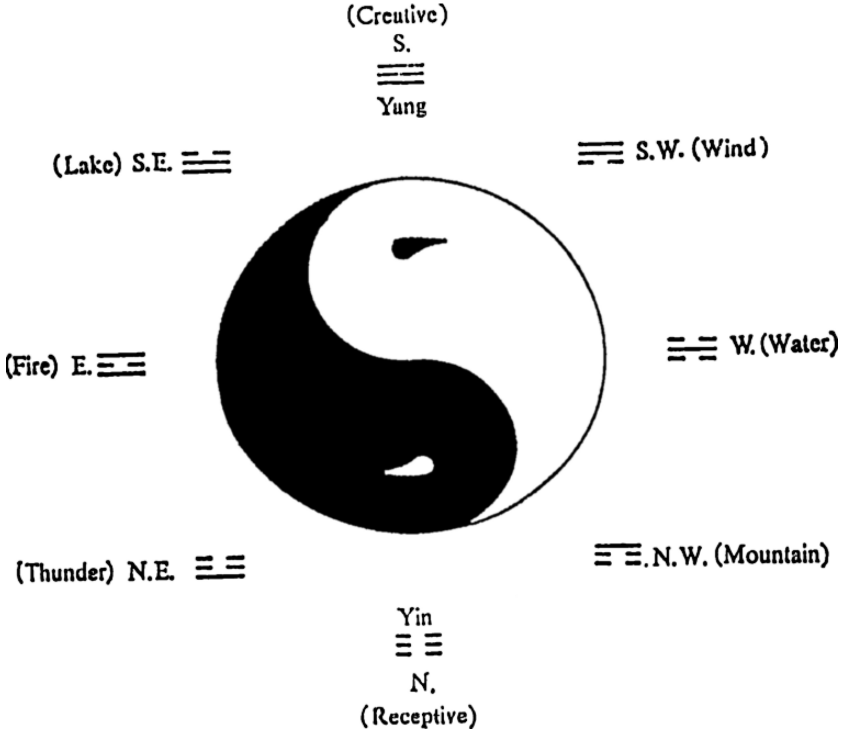

Fig. 1. Schematic representation of Yin/Yang theory

This hypothesis is mathematically expressed by the following model:

$$P = 100 + S\text{-}I \qquad (1)$$

where P represents the biological response to allelochemical, S and I are biological responses to the stimulatory and inhibitory attributes of the allelochemical respectively, and are expressed in the model by enzyme kinetics (An et al. 1993).

The illustration of the model is shown in Figs. 2–6. It can be seen from the illustrations that the model simulates the biological responses to allelochemicals and is in agreement with a wide range of experimental data taken from the literature. The model provides a useful platform for analysing experimental data, predicting allelopathic effects in practice, and for further exploring the fundamentals of allelopathy, which are discussed in the next sections.

9.3 Function of Allelopathy

One important concept, derived from the operation of the above dose-response model, is that a plant always contains a certain amount of allelochemical whether it is under stress or not.

Under normal conditions, allelochemicals in a plant may be inactive and concentrations may be relatively stable. However, as environment conditions become stressful for the plant, the allelochemical content dramatically increases. Stressful conditions include abnormal radiation (Koeppe et al. 1970; del Moral 1972), mineral deficiencies (Loche and Chouteau 1963; Lehman and Rice 1972), water deficits (del Moral 1972; Gilmore 1977), temperature extremes (Koeppe et al. 1970), and attack by pathogens and predators (Gayed and Rosa 1975; Woodhead 1981).

According to Cruickshank and Perrin (1964), a similar conclusion was proposed by Muller and Borger in 1939, i.e., ‘phytoalexin theory’ of disease resistance, which proposed that phytoalexins are metabolites that only form or become active when a parasite comes in contact with the host cells.

Plants have evolved means of adjusting the environment in their favour. It is well known that plants have physical means, such as the cuticle and trichomes, for their defense. Recently, it has been gradually recognized that allelochemicals may also be employed in the defense systems of plants (Levin 1971; Woodhead 1981; Lovett 1982; Lovett and Ryuntyu 1992; Bais et al. 2003). Plants may defend themselves by means of such chemicals in several ways. Phenolics, particularly flavonoids, are thought to protect plants from UV radiation (McClure 1975). Under stressful conditions, such as drought or insufficient nutrients, allelochemicals may inhibit the growth of other plants and favour the producer (Kuo et al. 1989). Phenolics and their oxidation products may affect fungal enzymes or the digestive enzymes of insects (Friend 1979; Woodhead 1981). Also, allelochemicals may cause the rapid death of a few cells in a plant under attack, and thus confine the pathogen and minimize the damage (Farkas and Kiraly 1962). Recently, by integrating ecological, physiological, biochemical, cellular, and genomic approaches Bais et al. (2003) demonstrated that *Centaurea maculosa* (spotted knapweed), an invasive species in the western United States, displaces native plant species by exuding the phototoxin (-)-catechin from its roots. This chemical triggers a wave of reactive oxygen species (ROS) in susceptible species, which is initiated at the root meristem and leads to a Ca^{2+} signaling cascade triggering genome-wide changes in gene expression and, ultimately, death of the root system.

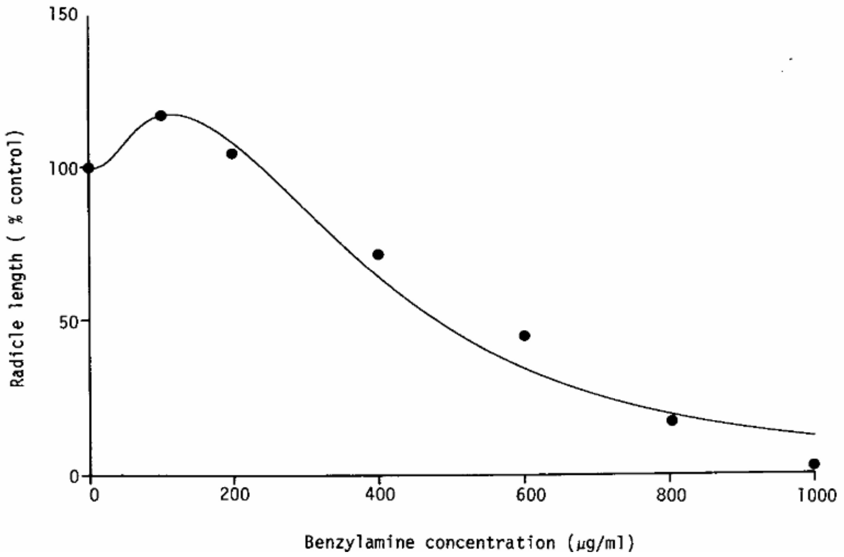

Fig. 2. The response of linseed in radicle length to benzylamine (An et al. 1993)

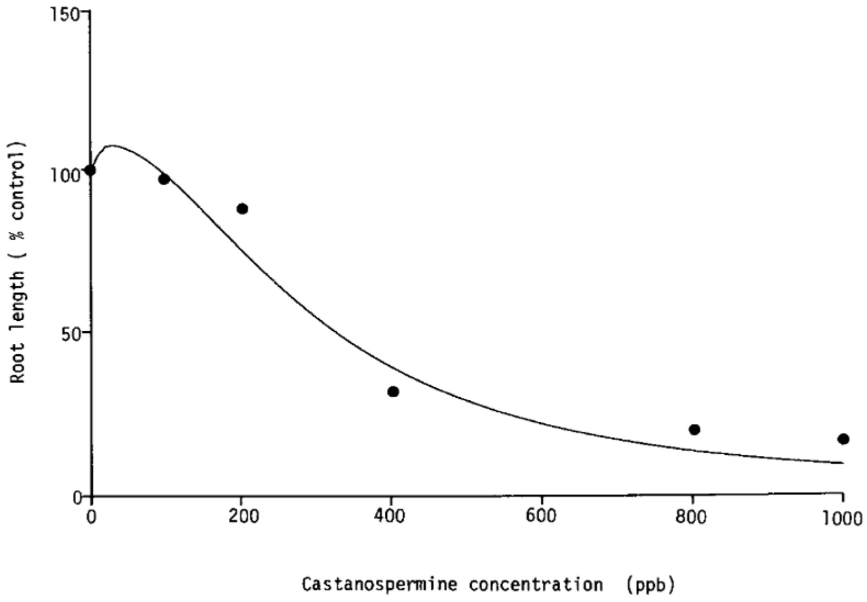

Fig. 3. Effect of castanospermine on root growth of lettuce (An et al. 1993)

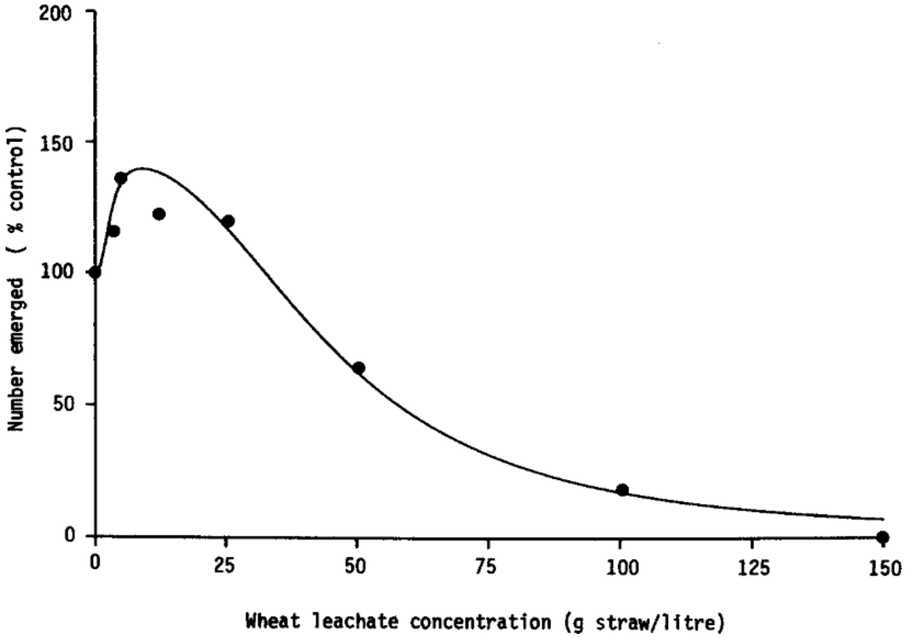

Fig. 4. The response of wild oats (*A. ludoviciana*) in total number emerged to wheat straw leachate (An et al. 1993)

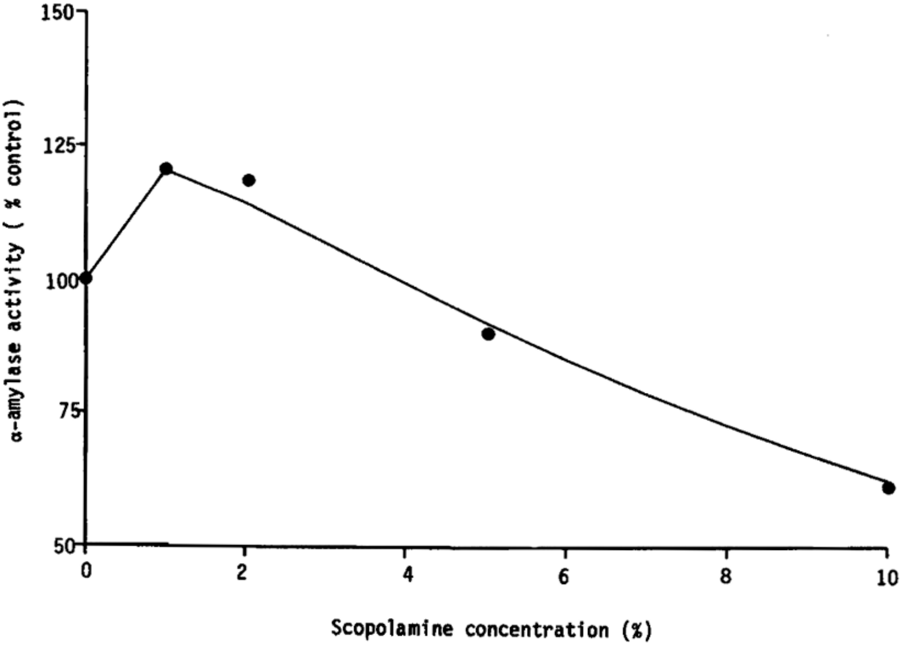

Fig. 5. The response of α-amylase activity to scopolamine (An et al. 1993)

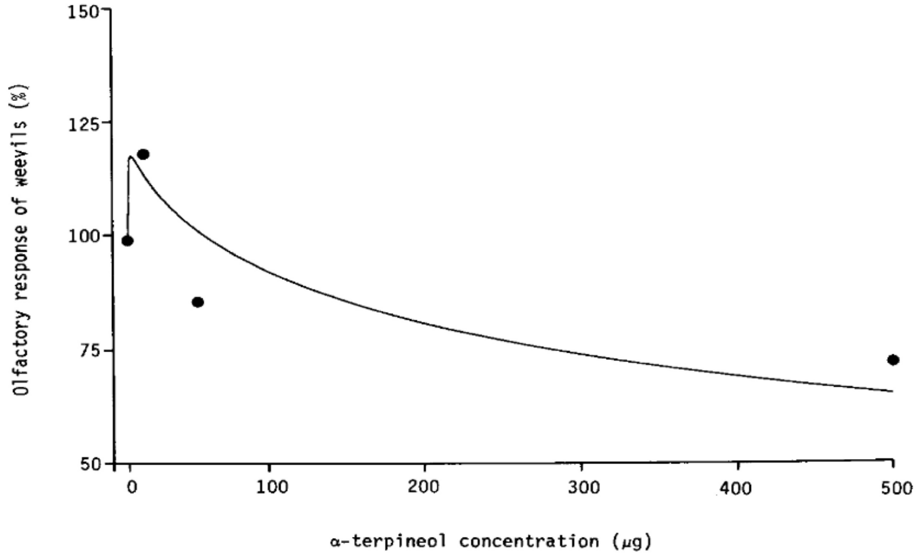

Fig. 6. Olfactory response of weevil larvae to α-terpineol (An et al. 1993)

It is conjectured that allelopathy may act as a defensive system in plants (Lovett and Ryuntyu 1992). Visible allelopathic effects or an increase of allelochemical contents in plants may be the result of the operation of this system under stress. Its purpose is to protect plants from stress and to keep an ideal or normal growth environment for plants. While under ideal conditions there is no allelopathic effect occurring, allelochemicals are inactive and their contents are stable. Such systems may be very complicated. It is known that plants produce numerous allelochemicals, each of which, or combinations of which may have different functions against stress factors. Stress, referred to here, has a broad definition that includes external constraints, such as water deficits, mineral deficiencies, temperature extremes, abnormal radiation, herbivores feeding and disease, etc., that disturbs normal plant growth and reduces the rate of dry matter production.

Allelopathy may have two functions in a plant: phytotoxic and autotoxic. Within the range of its capability to overcome stress, allelopathy is inhibitory to others except a producer. Under severe stress, beyond the adjusting capability of the plant, such as massive attack by pathogens or severe mineral deficiencies, allelopathy may act autotoxically to decrease the population of the producer; this may be the best survival strategy for plants under unfavourable conditions (Chou 1989). It has been observed that residues from plants grown under stressful conditions are more highly phytotoxic than under normal conditions (Mason-Sedun and Jessop 1989). Chou (1983) also noted that the roots of rice seedlings under waterlogged and oxygen-deficient conditions developed abnormally, and the plants tended to produce growth-inhibiting substances, resulting in decrease of yields. When the unfavourable conditions were removed by providing a good drainage system so that phytotoxins could be leached out of the soil, a significant yield increase of up to 40% was observed.

It is well documented that the concentration of secondary compounds in plant tissue is determined by the plant's genetic make-up in combination with its interaction with environmental conditions during growth (Bell and Charlwood 1980; Mason-Sedun 1986). Therefore, it is not surprising that allelopathic potentials, like other genetic characteristics, vary within and amongst species; this may reflect the extent of the plant's defense capability. For example, levels of phenolic acids in healthy plants of *Sorghum bicolor* differ considerably with cultivars. Cultivars with the highest normal phenolic levels are the most resistant to insect attack (Woodhead 1981).

Putnam and Tang (1986) and Lovett (1982, 1987) indicated that allelopathic characteristics are more likely to occur in crop predecessors or 'wild types' that have evolved in the presence of allelopathic and competitive influences from other species. If allelopathy acts as a defense reaction to stress, then humans' interference, such as irrigation, fertilizer and pesticide application, etc., may help to overcome stress for plants, and hence currently used cultivars have diminished or reduced allelopathic capacity.

Allelochemical content in plants is found to vary with experimental conditions. Woodhead (1981) reported that laboratory- and field-grown sorghum phenolics follow similar patterns, but that values for all field-grown plants are much higher than for the corresponding laboratory plants. This may be taken to imply that the ideal environment for plant growth is relative, and plants are always under some degree of stress. For example, Dicosmo and Towers (1984) pointed out that in plant cell cultures, altered secondary metabolism implies some kind of stress even when conditions seem to be optimum. Even when under no apparent stress, plants may contain a certain amount of allelochemical. The equilibrium point, at which no allelopathic effects occur (i.e., when stimulation and inhibition are equal), is likely to vary with growth conditions. Therefore, it is not surprising that allelochemical concentrations at the equilibrium point of one condition may show allelopathic effect on the same test species under different conditions. This may help to explain the argument that allelopathic effects are observed under conditions of no stress.

9.4 Periodic Production of Allelochemicals and Stress

Defense agents, allelochemicals or allelopathins, are largely classified as secondary plant metabolites that play an important role in allelopathic interactions or plant defenses and act as important ecological mechanisms (Rice 1984). The allelopathic characteristic of an allelochemical is defined as the biological property of the allelochemical as opposed to its physical or chemical properties (An et al. 1993). Allelochemicals are present in virtually all plant tissues, including leaves, flowers, fruits, stems, roots, rhizomes, seeds and pollen. They may be released from plants into the environment by means of volatilization, leaching, root exudation, and decomposition of plant residues (Putnam and Tang 1986). The contents of allelochemicals in living plants, and their fate and dynamics in environment are an important aspect in the study of allelopathy. Production of allelochemicals in living plants is affected by abiotic and biotic factors, which ultimately affect a plant's defense capability or allelopathic potential (Hedin 1990; Lovett and Hoult 1995; Einhellig 1995). Various research efforts have been made in identification of allelochemicals from living plants (Blua and Hanscom 1986; Niemeyer 1988; Wu et al. 2001), testing biological activities of allelochemicals identified (Waller et al. 1995; An et al. 2001), detecting their dynamics in plants and environment (Woodhead and Bernays 1978; Wolfson and Murdock 1990), characterizing their modes of actions (Wink and Bruning 1995; Czarnota et al. 2001), determining the effect of abiotic and biotic factors (Koeppe et al. 1970; Waterman et al. 1984; Einhellig 1989), and identifying their genetic make-up (Niemeyer and Jerez 1997; Quader et al. 2001), etc.

An et al. (2003) developed a mechanistic model, by applying the concept of diffusion process, that integrated scattered research information and present knowledge, assembled a generalized picture of allelochemical production in living plants, the fate of allelochemicals and their dynamics in the environment, and explored its possible ecological significance in plant allelopathy. Through their modelling work it is proposed that there are two kinds of allelochemical productions in a plant, which are dictated by age and plant stress, and are reflected by the corresponding dynamics in the environment. Generally, allelochemical content in living plants declines with age, and there is a corresponding fate in the environment, while periodic production may be a special case (An et al. 2003). By combing this model with the above mentioned dose-response model in Section 9.2 they demonstrated the possible existence of corresponding periodic dynamics in the environment, successfully simulated response fluctuation of receiver plants to allelochemicals, theoretically interpreted such results reported in the literature, and contributed it to the periodic production of allelochemicals (Figs. 7–8). This helped us to understand why the results of allelopathic research are disparate when conducted under different stages of plant growth and development, and why results fluctuate as experiments proceed, and helped to reduce confusion on allelopathy and suggest directions for future research.

It is well documented that the production of secondary plant compounds in plant tissue is determined by the plant's genetic make-up in combination with its interaction with environmental conditions during growth (Bell and Charlwood 1980; Lovett 1982; Mason-Sedun 1986; Niemeyer and Jerez 1997; Quader et al. 2001). Considering that allelopathy acts as a defense system in a plant, the observation that the overall concentration of allelochemicals in plants declines with increasing age of the plant is logical. A defense system is an inherent character of the plant. Like other genetic characters, as the age of a plant increases, its defense capability inevitably experiences weak, strong, and then again weakening stages.

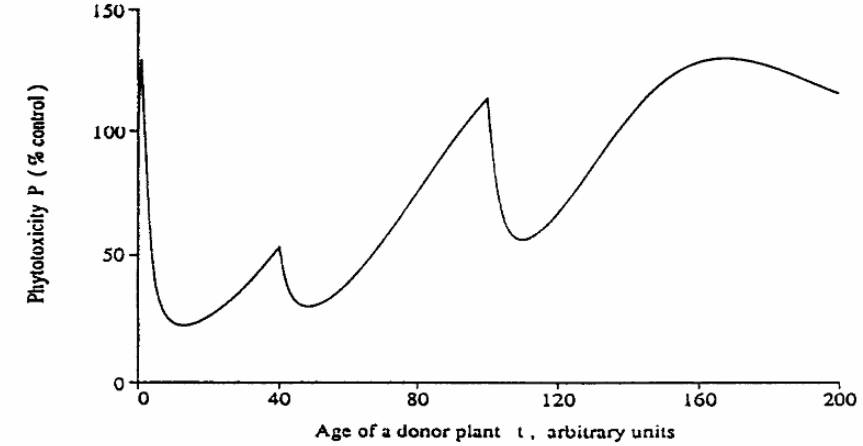

Fig. 7. Simulated fluctuation of responses of receiver plants to periodic dynamics of allelochemicals in the environment, which is described by the combination of allelochemical – biological response model (An et al. 1993) and the model (An et al. 2003). *P* is response of test plants to allelochemicals, % control; *t* is time course of donor plant growth, arbitrary unit (An et al. 2003)

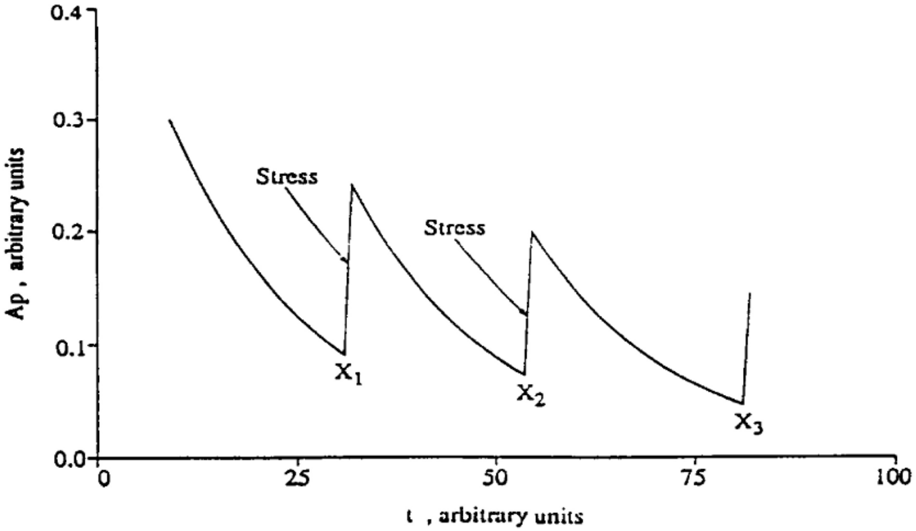

Fig. 8. Simulated periodic production of an allelochemical under constant stress. $A\mathrm{p}$ is allelochemical concentration in plant; *t* is time course of plant growth, both in arbitrary units. $X1, X2, X3$ are concentrations at an equilibrium (An et al. 2003)

A seed will have inherited characters, but also will be physically conferred a certain amount of allelochemical from a parent, in the same way that starch and protein are transferred into the endosperm. For example, Mothes (1955) showed that the alkaloid content of *D. stramonium* seeds increased significantly as the seeds ripened. At the beginning of germination, a plant has to use these allelochemicals to inhibit other species in order to favour its own germination (e.g. alkaloids, Lovett et al. 1981), due to the fact that its inherent defense capability has not built up, just as germinating seeds have to use storage materials conferred from a parent before its photosynthesis system operates. Soon after germination, all systems of a plant gradually begin to operate. For example, at the sixth day of germination, seedlings of *D. stramonium* are capable of synthesizing phytotoxic alkaloids and releasing them into the environment (James 1946; Mothes 1955). As a plant becomes mature, the capability of producing allelochemicals declines in the same way as other characters of a plant. Allelopathic potential, or allelochemical production, reflects the extent of the defence capability.

As environment conditions become stressful for plant growth, the allelochemical content in a plant increases. This phenomenon, together with the periodic production of allelochemicals, may be viewed as a response of plant chemical defense system to stress. Growth and development of a plant requires a normal or ideal environment while stress is constant and indeterminate, in other words, a plant is always surrounded by stress. In order to optimize growth, a plant is constantly attempting to overcome stress. It may be that once stress occurs, a plant immediately responds by producing allelochemicals, which may result in the overcoming or inhibiting of stress. After that, allelochemical content declines, allowing the stress to affect the plant again. This means that stress may occur again, and the plant responds by releasing allelochemicals again, which shows a periodic pattern over a time course (Fig. 8). Allelochemical levels in plants may generally decrease with the age of plants, while during the whole time course of plant growth, allelochemical levels may fluctuate as a defense reaction to stress. The timing and extent of periodic production may depend on the frequency of stress occurring, its extent and the defense capability of a plant. Since stress is less predictable and indeterminate in the field, presumably, the same pattern of periodic production is unlikely to be experimentally repeated.

9.5 Allelopathy and Competition

Rice (1984) clearly distinguishes allelopathy from competition, saying that the effects of allelopathy depend on a chemical compound being added to the environment, whereas competition involves the removal or reduction of some factors from the environment that is required by some other plant sharing the habitat. However, as argued earlier in this section, a plant may employ allelopathy as a defense strategy and its purpose is to relieve plants from stress and keep a normal or optimum environment for plant growth. Therefore, it can be argued that allelopathy and competition are not disparate processes, there is some link, at least, between them. This view accords with that of Harper (1977), namely that allelopathy and competition are parts of the complex of 'interference' which takes place between plants. In fact many attempts were made to estimate the relative contributions of allelopathy and competition to interference, e.g. allelopathy contributed to 35% reduction of corn yield in a total 90% reduction by interference (Bell and Koeppe 1972); 19–69% was attributable to allelopathy in 28–95% of interference by fescue with sweetgum (Walters and Gilmore 1976). With a series of carefully designed experiments and subsequent modelling, Liu et al. (2005) assessed the contribution of competition and allelopathy to the total interference by barley on white mustard and reported that allelopathy contributed 37–43% of total interference, while competition contributed to the 63–57% of interference.

As plants inhabit stressful environments such as those characterized by drought, severe diseases, high UV radiation, limited space and insufficient nutrients, they may exhibit structural and biochemical changes favouring their survival. Biochemically, many plants produce toxic chemicals which inhibit the growth of other plants (Kuo et al. 1989). Chou (1989) claimed that perhaps the production of phytotoxins can be interpreted as an adaptative strategy aimed at suppressing the growth of competitive plants that utilize the same nutrients. Conventional ecological perspective has often attributed a species' invasiveness mainly to enhanced resource competition after escape from natural enemies. However, recent findings by Bais et al. (2003) suggest that biochemical potential of the plant, i.e., allelopathy, may play an important determinant role in successful invasion by certain plant species. By integrating ecological, physiological, biochemical, cellular, and genomic approaches they demonstrated that *Centaurea maculosa* (spotted knapweed), an invasive species in the western United States, displaces native plant species by exuding the phototoxin (-)-catechin from its roots, and the inhibition of native species' growth and germination in field soils is at natural concentrations of (-)-catechin.

Competition may result when two plants draw on a mutual requirement (e.g. nitrogen) so that the supply of this requirement to at least one of the plants is decreased by the presence of the other. This reduced supply may then affect growth, reproduction, or ultimately, even survival of the plant (Donald, 1963). Thus, significant point of competition is that the immediate supply of a common resource falls below the combined demands of the plants. This can be defined as a kind of stress.

A plant competes to supply itself with the necessary factors for growth. To do so in an environment of insufficient supply, a simple way is to inhibit the growth of competitive plants, and thereby decrease consumption of limited resources by those competitors. There arises a question: 'what means can a plant employ for doing so?'. From an ecological and evolutionary point of view there are several possibilities. It has been documented that allelopathic effect can be enhanced by stress (Einhellig 1989; Hartung and Stephens 1983). This means that the receiver plant under stress, such as mineral deficiencies, would suffer from both the phytotoxic effects of allelochemicals and the stress of nutrient deficiency, and so its growth would be inhibited. This suggests, therefore, that one effective means for a plant to take is to produce biochemically phytotoxic substances and release them into the shared habitat to inhibit competitive plants, a strategy similar to the 'novel weapons hypothesis' (Bais et al. 2003). This can be evidenced by the fact that competition is often associated with allelopathy, e.g. plants such as barley which have strong competitive ability are often associated with strong allelopathic potential (Overland 1966), but not vice verse, e.g. in response to massive attack by insects. This may further indicate the fact of a link between allelopathy and competition.

Clement et al. (1929) stated, 'in the exact sense, two plants, no matter how close, do not compete with each other so long as the water content, the nutrient material, the light and the heat are in excess of the needs of both.' While under such conditions allelopathy may still occur. Indeed, Wu et al. (2000) experimentally demonstrated such a situation by developing a laboratory screening bioassay called 'equal compartment agar method' (ECAM). When deployed to assess wheat-seedling allelopathy on annual ryegrass this method successfully precluded resource competition between donor and receiver seedlings. The setup of this bioassay allowed allelochemicals released from the living donor roots being diffused into the growth agar medium to affect the root growth of the receiver plants, and the continuous growth of donor seedlings ensured a constant release and accumulation of allelochemicals into the growth medium, which simulated the continuous growth in nature (Wu et al. 2001). This bioassay was aseptically managed so that microbial involvement was also avoided. The screening results obtained could be attributed solely to the allelopathic effect of donor seedlings (Wu et al. 2001).

It is therefore proposed that allelopathy may be one of the internal motive forces of a plant for defending and maintaining a favourable growth environment, while competition is only part of the external expression of such operation. Strong or weak competitive capability of a plant may be one kind of measurement of allelopathic potential, but not merely one.

Allelopathic potential may be an inherent character of a plant (in plant communities), and it is not necessarily the case that in competing plant communities one component is inhibited while the other benefits. It may be that both sides are affected, though not necessarily to the same extent, and equilibrium may be reached between them during competition for a common resource.

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Part 3 Application of Allelopathy in Agriculture and Forestry

10. Progress and Prospect of Rice Allelopathy Research

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Abstract. An improvement in allelopathic potential in rice will have a great impact on both low-input and high-input management systems. Alleopathy alone is not likely to replace other weed control practices, but it surely functions as a component of integrated weed management technology. Marginally reduced use of herbicide over time will provide significant economical benefits to farmers and will also reduce adverse effects on the environment. There is a great possibility of breeding new allelopathic rice by regulating their capacity to produce allelopathic substances. Allelopathic potential in rice was greatly improved by the classical breeding method of crossing between a non-allelopathic commercial cultivar and an allelopathic cultivar. The first priority research areas are identification of allelochemicals and genes responsible for allelopathic effects and these traits can be easily incorporated into improved cultivars by either classical breeding or biotechnology. Allelopathic activities of rice seem to be derived from the joint action of momilactones with phenolic compounds. Phenolics, however, are unlikely to be the primary allelochemicals since they never reach a phytotoxic level in soil. However, it is believed that phenolics like other multiple compounds present at concentrations below the activity threshold could have pronounced allelopathic effects through their joint action. Momilactone B seems to be the responsible allelochemical because its activities were 30-fold to 300-fold greater than phenolic acids. Insertion of two specific genes such as CA4H for p-coumaric acid and OsDTS2 for momilactone into one commercial rice cultivar by biotechnology will lead to the development of allelopathic rice cultivar. Further studies on genetic control of rice allelopathy, the complete array of the responsible chemicals in rice root exudates and their mode of action will shed more light on developing an allelopathic rice crop.

10.1 Introduction

Allelopathy is a natural phenomenon that refers to any direct or indirect harmful or beneficial effect by one plant (or microorganism) on another through the production of chemical compounds deposited into the environment. Crop plants have the capability to produce and excrete allelochemicals into their surrounding environment to inhibit the growth of weeds in their vicinity.

Dekker and Meggitt (1983) suggested that most allelochemicals are released from the early developmental stage of plants when the plants are most stressed and competing with neighboring plants for light, nutrients and water. Thus, allelopathy can be applicable as a component of an integrated weed management program.

The chemicals concerned in higher plant interaction, called allelopahtic substances or toxins, are typical secondary metabolites and appear to be mainly low molecular weight compounds of relatively simple structure. Most allelochemicls that have been positively identified are either volatile terpenes or phenolic compounds (Harborne 1993). There are a number of papers on phenolics (Rice 1987; Chou et al. 1991; Inderjit 1996; Blum 1996; Mattice et al. 1998; Olofsdotter et al. 2002; Seal et al. 2004a,b) and momilactones (Nojiri et al. 1996; Araki and Kurahashi 1999; Tamogami and Kodama 2000; Kato-Noguchi and Ino 2005) as rice allelochemicals. Therefore, it is postulated that allelopathic activities of rice may be derived from a joint action of momilactones with phenolic compounds and some unknown compounds. There is also a possibility that the responsible compounds have not been identified and key compounds remain undiscovered (Seal et al. 2004b), since less than 5% of the secondary metabolites from plants have been isolated and identified, which leaves a broad scope for possible involvement of other compounds (Worsham 1989).

Recently, the allelopathic potential of rice has received a great deal of attention since Dilday et al. (1991) identified rice cultivars exhibiting allelopathic potential against ducksalad [*Heteranthera limosa* (Sw.) Willd]. Rice allelopathy has been made a subject of continued research for a decade and progress has been made in a range of fields (Olofsdotter et al. 1995). In addition, allelopathic potential has been also reported from numerous crops like barley (Lovett and Hoult 1995), cucumber (Putnam and Duke 1974), oats (Fay and Duke 1977), rice (Dilday et al. 1998), sorghum (Nimbal et al. 1996), sunflower (Leather 1983), tobacco (Patrick et al. 1963) and wheat (Wu et al. 1999).

There is an increasing problem with herbicide resistant weeds, which could be solved by highly allelopathic cultivars (Lemerle et al. 2001). Modern cultivars that combine early vigor and high specific allelopathic activity should therefore be selected to allow for an effective weed management with reduced herbicide input (Bertholdsson 2005).

The possibility of incorporating allelopathic traits into improved rice cultivars, thus reducing the need for applying herbicides to the crop is worth exploring (Khush 1996). Selection for superior genotypes with allelopathic potential has been carried out in several crops, and there is accumulated evidence that crop varieties differ significantly in their ability to suppress the growth of certain weed species (Wu et al. 1999). However, so far, no commercial cultivars carrying allelopathic properties have been developed (Duke et al. 2001). Recently, Ma et al. (2006) reported that a potent allelopathic rice cultivar was made by traditional breeding method.

Once the allelopathic genes are identified, the genes could be incorporated into modern cultivars through either genetic manipulation or classical breeding method. Biotechnology could be used not only to maintain but also to increase the chemical diversity of plant secondary metabolites that defend the crop. This would allow the production of a crop that can produce several different natural chemicals. Duke (2005) suggested that improvement of allelopathic traits of crops through genetic manipulation is a formidable task and the added-allelopathic trait is unlikely to completely replace other weed control technology.

There are a number of review papers on allelopathy related to rice crop (Duke et al. 2001; Olofsdotter 2001a,b; Kim and Shin 2003, 2005).

This paper is mainly focused on reviewing allelochemicals related to rice allelopathy, achievement in breeding allelopathic rice and the genetic transfer of allelopahic traits.

10.2 Allelopathic Traits

10.2.1 Morphological Characters

What are allelopathic traits? Morphological characteristics such as early seedling emergence, seedling vigor, fast growth rates that produce a dense canopy, greater plant height, greater root volume and longer growth duration are known to increase the ability of rice cultivars to compete with weeds (Minotti and Sweet 1981; Berkowitz 1988).

In rice, plant traits such as longer duration of the rice crop, earlier seedling emergence and vigor, faster growing rates, higher tillering capacity, taller plants and greater root volume have been traditionally considered to be related to competitive ability (Khush 1996). However, some of these traits (e.g. taller plant and high tillering capacity) oppose current breeding efforts for a new high-yielding plant type (Olofsdotter 2001a).

Bertholdsson (2005) revealed that the early crop biomass and potential allelopathic activity were the only parameters that significantly contributed to competitiveness in both barley and wheat, determined by multiple regression analysis. Plant height is often described as one of the most important factors in the total competitive ability of a crop and accounts for a similar percentage of total competitive ability (Gaudet and Keddy 1988). Jung et al. (2006) revealed possible relation of color of rice awns with allelopathic activity.

Olofsdotter et al. (1999) and Seal et al. (2005) demonstrated that there is a positive correlation between allelopathic potential measured in the laboratory screening and actual performance in the field. The results suggest that using allelopathy as part of an integrated weed management might be possible. Olofsdotter et al. (1995) also reported that allelopathic traits might be polygenic in nature weakly responded with yield or other important agronomic features, thereby, making it difficult in breeding allelopathic rice varieties.

According to Olofsdotter et al. (1999), it is certain that visible morphological characters or any trait for proving allelopathy are not presently available….However, it is obvious that weed-suppressing cultivars have either superior competitive ability, allelopathic potential, or both of these traits. Further work on the genetic background of both competitive ability and allelopathy will shed more light on understanding the weed-suppressing ability of crops.

10.2.2 Screening Methods

To evaluate rice allelopathic potential, several methods such as the stairstep method (Bonner 1950; Liu and Lovett 1993), hydroponics culture test (Einhellig et al. 1985), relay-seeding technique (Navarez and Olofsdotter 1996), agar medium test (Fujii 1992; Wu et al. 1999), cluster analysis using HPLC (Mattice et al. 2001), water extract method (Kim et al. 1999; Ebana et al. 2001), 24-well plate bioassay (Rimando et al. 2001) and bioactivity-guided isolation method which can be automated with modern instruments such as liquid chromatography-mass spectrometry (LC/MS) with Nuclear Magnetic Resonance (Duke et al. 2002) have been introduced. Another valuable screening method may be the direct field test, although it is difficult to dealing with a large number of germplasm. Each method has advantages and disadvantages.

It is believed that a great progress has been achieved so far in screening, but considering the same rice cultivar shows different responses depending upon the screening method employed, it is worthwhile to develop an universal bioassay method (easy to carry out, but most reliable and economic one) applicable to all the conditions.

10.3 Genetic Variability

10.3.1 Variation in Allelopathic Potential

Dilday et al. (1998) identified 412 accessions having allelopathic potential against ducksalad [*Heteranthera limosa* (Sw.) Willd] among 12,000 accessions that originated from 31 different countries. These accessions were genetically very diverse, indicating that allelopathic accessions are widely present in rice germplasm. There are a number of papers related to different rice allelopathic potentials among rice germplasm (Dilday et al. 1991, 1998; Fujii 1992; Olofsdotter et al. 1995; Kim et al. 1999; Seal et al. 2005). The existence of varied allelopathic potentials offers a genetic pool for selection of crop cultivars with high allelopathic ability (Wu et al. 1999).

For instance, tropical japonica rice varieties have shown greater allelopathic potential against weeds, especially *Echinochloa* spp. than other rice varieties (Jensen et al. 2001).

10.3.2 Quantitative Trait Loci Analysis

There is very limited knowledge on allelopathy genetics. Moreover, an international breeding effort has not been made to genetically improve the allelopathic potential of crops, mainly due to poor knowledge on this phenomenon.

Quantitative trait loci (QTL) analysis is the initial step in the genetic analysis of rice allelopathy. If genes of interest have been located, it is possible to identify molecular markers closely linked to those genes. Identification of QTLs for allelopathic effect is useful to provide basic information for selection of highly allelopathic rice cultivars. In this regard, a recent work conducted by Jensen et al. (2001) would be very valuable; QTL mapping using 142 DNA markers were located in 142 recombinant inbred lines derived from a cross between cultivar IAC 165 (japonica upland variety), which has strong allelopathic potential and cultivar CO 39 (indica irrigated variety), which has weak allelopathic potential. Three main loci, each accounting for about 10% of the upregulation of allelochemical production were localized in rice chromosomes 2 and 3. The two QTL traits on chromosome 3 were closely linked, so they could easily be manipulated.

Another work done by Okuno and Ebana (2003) revealed that seven QTLs controlling allelopathic effect of rice were on chromosome 1, 3, 5, 6, 7, 11 and 12. Zeng et al. (2003) detected four QTLs from a typical Indica and Japonica hybrid correlating to allelopathy on chromosome 3, 9, 10 and 12. It was suggested that allelopathic effect in rice is influenced by a complex quantitative trait and that the QTLs associated with allelopathy differed with the rice cultivars used.

Genetic mapping of QTLs has helped our understanding of the genes involved in the inheritance of quantitative traits. The use of restriction fragment length polymorphism (RFLP) markers has increased the efficiency of mapping QTLs, because of the greater number of makers that can be scored in a single population relative to other markers such as isozymes or morphological makers (Wu et al. 1999). There are a number of papers on the successful use of RFLP technique to locate QTLs conferring the production of phytochemicals in various crops. The similarities between allelopathic agents and these phytochemical-based traits make it possible to use the same research methodology to investigate the genetic control of the allelochemical production in crops (Wu et al. 1999). This area is still in its infancy. Further work along this line will provide a great opportunity for more understanding of the genetic control of rice allelopathy.

10.4 Rice Allelochemicals

10.4.1 Synthesis of Allelochemicals by Environmental Stresses

Until recently, many studies verified the mechanisms of a self-defense system, including allelopathy in plants, particularly phenylpropanoid (Fig. 1) and isoterpenoid metabolism (Fig. 2).

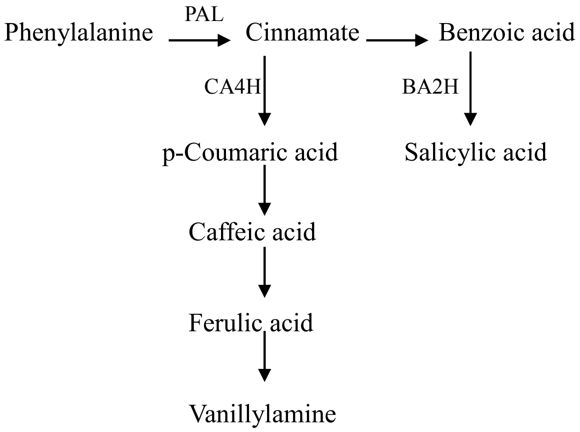
Plants respond to environmental stresses through a variety of biochemical reactions. The increase of phenolic and isoterpenoid compounds under environmental stresses has been well documented. For example, enhanced UV-B light induces the accumulation of phenylpropanoids and flavonoids in different plant species, such as bean, parsley, potato, tomato, maize, rye, barley and rice (Hahlbrock and Scheel 1989; Ballare et al. 1995; Tevini et al. 1991; Liu et al. 1995; Kim et al. 2000a).

10.4.2 Pathways for Allelochemical Synthesis

Phenolics and terpenes are two important groups of secondary metabolites, that receive a great deal of attention as alleleochemicals (Harborne 1993).

Phenolic compounds are synthesized by the phenylpropanoid pathway (Fig. 1). All phenylpropanoids are derived from cinnamic acid, which is formed from phenylalanine by the catalytic action of phenylalanine ammonia-lyase (PAL), the branch point enzyme between primary (shikimate pathway) and secondary (phenylpropanoid) metabolism. Many phenolic compounds not only have a physiologically functional ability, but also plant allelopathic potential.

Terpenoid compounds are produced from C_5 isoprenoid units and the classification of different families of isoprenoids is based on the number of C_5 isoprenoid units present in the skeleton of compounds (Gershenzon and Croteau 1993). In particular, diterpenoids are known to play an important role in the self-defense mechanism and allelopathic potential in rice plant. Momilactones and oryzalexin A are derived form diterpenoids (Fig. 2).

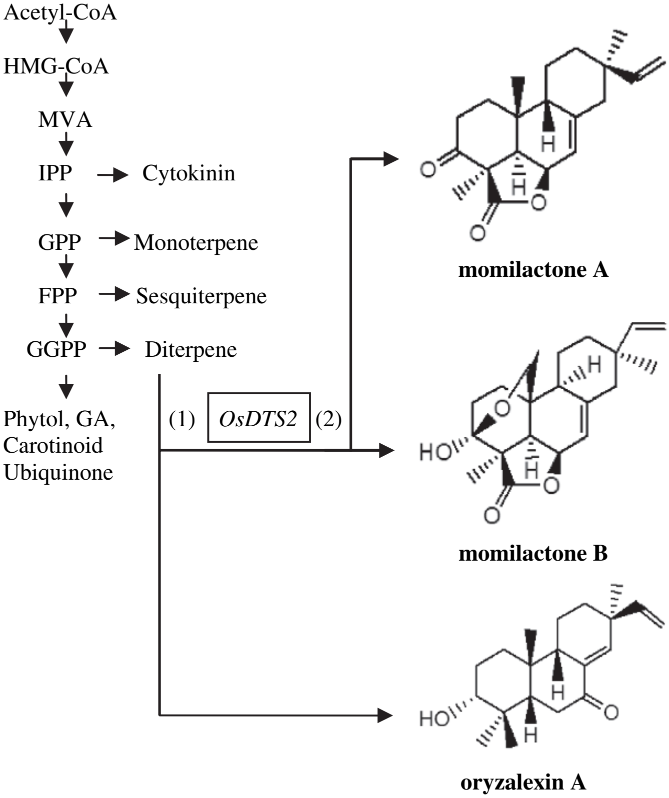


PAL: phenylalanine ammonia-lyase

CA4H: cinnamic acid 4-hydroxylase

BA2H: benzoic acid 2-hydroxylase

Fig. 1. Scheme of phenylpropanoid pathway



- IPP: Isopentyl pyrophosphate
- GPP: Geranyl pyrophosphate
- FPP: Farnesyl pyrophosphate
- GGPP: Geranylgeranyl-PP
- OsDTS2: *syn* - copalyl diphosphate specific 9β – pimara -7,15-diene synthase to convert *syn* - cpp (1) to *syn* – pimara – 7, 15 diene, (2)a precursor of momilactone A and B

Fig. 2. Scheme of isoprenoid pathway

Terpenoids play diverse functions in plants as hormones (gibberellins, abscisic acid), photosynthetic pigments (phytol, carotenoids), electron carriers (ubiquinone, plastophates), mediators of polysaccharide assembly (polyprenyl phosphates), structural components of membranes (phytosterols), and agents in communication and defense.

10.4.3 Putative Allelochemicals

Studies on rice allelochemicals have been conducted by various researchers (Chung et al. 2001; Kato-Noguchi et al. 2002; Kim and Kim 2000; Kim and Kim 2002; Rimando et al. 2001; Kong et al. 2004a,b; Seal et al. 2004a,b). Seal et al. (2004b) found that a typical chromatogram of rice root exudates contains about 200 peaks, which are potentially denoting at least 200 different compounds. Which of those chemicals plays a key role in the observed allelopathic effect? Seal et al. (2004a) isolated and identified twenty-five compounds from rice root exudates. They were classified into three chemical classes such as phenolics, phenylalkanoic acids and indoles.

Several classes of secondary metabolites determined from rice root exudates include phenolics (Jung et al. 2001; Kim and Kim 2002; Mattice et al. 1998; Rimando et al. 2001; Olofsdotter et al. 2002; Inderjit et al. 2002; Seal et al. 2004a,b), alkyl resocinols (Bouillant et al. 1994), momilactone B (Kato-Noguchi et al. 2002; Kato-Noguchi and Ino 2003, 2005; Kong et al. 2004b), carbohydrates and amino acids (Bacilio-Jimenez et al. 2003), and flavones (Kong et al. 2004b).

Kim et al. (2000b) identified several compounds by GC/MS analysis from Kouketsumochi, a potent allelopathic rice, such as sterols, benzaldehydes, benzene derivatives, long chain fatty acid esters, aldehydes, ketones and amines from fractions with biological activity. Several compounds from Taichung Native 1, an allelopathic rice, such as azelaic, *p*-coumaric acids, 1*H*-indole-carboxaldehyde, 1*H*-indole-3-carboxylic, 1*H*-indole-5-carboxylic acids and 1,2-benzenedicarboxylic acid bis (2-ethylhexyl) ester have been identified by the bioassay-guided isolation method (Rimando et al. 2001). It is postulated that allelopathic effects of rice may be induced by a combination and interaction of a complex mixture of compounds.

10.4.4 Phenolics

There are a number of studies indicating that common putative allelochemicals found in rice were phenolic compounds (Rice 1987; Chou et al. 1991; Inderjit 1996; Mattice et al. 1998; Blum 1998). Olofsdotter et al. (2002) found that the maximum release rate of phenolic acids during the first month of growth was approximately 10 μg/plant/day, in other words, approximately 1 mg/m^2/day at a conventional plant density of 100 rice plant/m^2, which cannot provide concentrations close to phytotoxic levels determined for rice cultivars and weed species. Their results indicate that phenolics are unlikely to be the primary allelochemicals in rice since their concentrations in paddy never reach phytotoxic levels, but suggests that these results do not preclude the possibility that phenolic acids might be one component in a mixture of chemicals that, when present simultaneously, are allelopathic (Olofsdotter et al. 2002).

Seal et al. (2004a) quantified the levels of 15 compounds from rice root exudates using GC/MS/MS and found that six of the seven most abundant compounds isolated were phenolic acids. Great amounts of three of these six compounds such as trans-ferulic acid, p-hydrobenzoic acid and caffeic acid were detected in the exudates of allelopathic rice cultivars. Further they determined that five selected phenolics, namely caffeic, p-coumaric, p-hydrobenzoic, syringic and vanillic acids from rice exudates were best correlated with the observed allelopathic effect on arrowhead (*Sagittaria montevidensis*) root growth with multiple regression analysis (Seal et al. 2004b). Despite this positive correlation of phenolic acids, they reported similar results as Olofsdotter et al. (2002) that the amount of phenolic compounds quantified in the exudates was much lower than the required threshold concentration for arrowhead inhibition.

Whether allelopathy under field conditions results from a single compound or a mixture of several phytotoxins has been debated (Einhellig 1999). Studies of Blum (1996) on mixtures of phenolic compounds have shown that individual concentration can be additive when evaluating for phytotoxic affects. Further, Inderjit et al. (2002) found no evidence for synergistic activities of phenolic acids in the mixture, in a joint action study of the phenolic acids p-hydroxybenzoic, p-coumaric and ferulic acids on root growth inhibition of perennial ryegrasss (*Lolium perenne*).

In rice soils, p-coumaric acid among 13 different phenolic acids isolated was released in the highest amount during decomposition of rice straw (Kuwatsuka and Shindo 1973). Among the allelopathic substances identified, p-coumaric acid, a known allelochemical, inhibited the germination of lettuce (*Lactuca sativa*) seedlings at 1 mM, but was active against barnyardgrass only at concentrations higher than 3 mM (Rimando et al. 2001). Kim et al. (2000a) found that p-coumaric acid identified in extracts of allelopathic rice cultivars such as Kouketsumochi, Tang Gan and Taichung Native 1 was markedly increased under UV irradiation. The results above strongly suggest that p-coumaric acid might be a compound regulated by environmental stresses and one of the important phenolics in rice allelopathy.

Several phenolic acids such as p-coumaric acid, p-hydrobenzoic acid, feruic acid and vanillic acid have also been detected and received a great deal of attention as rice allelochemicals (Chou and Chiou 1979; Chou et al. 1991; Chung et al. 2001; Rimando et al. 2001; Seal et al. 2004a,b). However, the problem is relatively weak growth inhibitory activities of phenolics, requiring concentrations greater than 1 mmol L^{-1} to inhibit the test plants (Dalton 1999). Nonetheless, it is believed that phenolics must play an important role for rice allelopathy.

Phenolic acids have essentially the same site of action and thus, the action could never be synergistic unless the sites were different or the compounds interact to form a new compound (Inderjit et al. 2002). Einhellig (1995) proposed membrane-associated disturbance as the common mode of action of phenolic acids. After their entry through the membrane, phenolic acids caused depolarization of the cell membrane influencing membrane ATPase activity and affecting ion flux and retention (Einhellig 1995). Some phenolic acids like p-hydroxybenzoic, p-coumaric and ferulic acids inhibit hydraulic conductivity and nutrient uptake by plant roots, resulting in growth inhibition (Blum 1995).

Phenolic acids are the major allelochemicals implicated in crop allelopathy (Inderjit 1996), but considering their concentrations never reach phytotoxic levels, more thorough studies are needed to understand the role of phenolics in rice allelopathy.

10.4.5 Terpenes

Kato et al. (1973) first isolated momilactone A and B from rice husk as growth inhibitors involved in seed dormancy. Both momilactones were later found in rice leaves and straw as phytoalexins (Cartwright et al. 1977; Kodama et al. 1988). The function of momilactone A as a phytoalexin has been extensively studied by Nojiri et al. (1996), Araki and Kurahashi (1999), Tamogami and Kodama (2000). Momilactone B was known as a more active inhibitor than momilactonee A (Kato et al. 1973; Takahashi et al. 1976). Recently, Kato-Noguchi and Ino (2005) found momilactone B in shoots and roots of rice plants over their entire life cycle and the levels of momilactone B in both shoots and roots increased with rice plants grown until flower initiating stage, reaching 245 and 64.1 nmol g^{-1} fresh weight, respectively and then decreased.

Kato-Noguchi and Ino (2003) quantified 1.8 nmol of momilactone B per rice seedling (15-day old) from culture solution compared with endogenous levels of 0.32 and 0.63 nmol per root and shoot, respectively, indicating that 15-day old rice seedlings actively release momilactone B into culture solution. Momilactone B inhibited the growth of cress (*Lepidium sativum*) and lettuce (*Lactuca sativa*) seedlings at concentrations greater than 3 and 30 μM, respectively, suggesting that momilactone B may have a more important role in rice allelopathy (Kato-Noguchi and Ino 2005) since its activities were 30- to 300-fold greater than phenolic acids (Kato-Noguchi et al. 2002).

Recently, Jung et al. (2006) quantified momilactone A and B in rice hull of 99 varieties. Out of these varieties, momilactone A was identified in 66 varieties and momilactone B in 58 varieties. The average amount of momilactones in rice varieties was 4.9 $\mu g\ g^{-1}$ for momolactone A and 2.9 $\mu g\ g^{-1}$ for momilactone B. They suggested that theoretically, it needed about 14,540 kg of rice hulls per hectare to give an inhibitory effect on paddy weeds. Thus, it is postulated that application of rice hull in huge volume may be practically impossible for weed control.

Another interesting result is that momilactone A caused a greater inhibitory effect than momilactone B, which is contrary to the works of Kato et al. (1973), and Kato-Noguchi and Ino (2005) who reported that inhibitory effect of momilactone B was much greater than that of momilactone A. Jung et al. (2006) explained that difference in inhibitory effect might be due to interactions of compounds or different sensitivity of testing plants between laboratory and field conditions.

However, this may not be enough to explain the different activity caused by momilactone A and B. The works of Kato-Noguchi and Ino (2005) might be more relevant because they determined momilactones directly from rice root exudates.

However, less is understood about the mode of action of momilactones. Therefore, further studies on the mode of action of momilactones will help explain the role of momilactone A and B in rice allelopathy.

10.5 Breeding of Allelopathic Rice

10.5.1 Need for Allelopathic Rice

A number of rice cultivars or accessions having allelopathic potential have been identified (Dilday et al. 1991; Fujii 1992; Kim and Shin 1998; Olofsdotter et al. 1999). However, no commercial allelopathic rice cultivars have been developed yet. As indicated by Khush (1996), it may be worthwhile to incorporate allelopathic potential, as a parameter in breeding programs.

In order to implement this technology, the anticipated problems of autotoxicity, the residual effects of allelopathic cultivars and the tolerance of weed populations upon repeated cultivation of allelopathic cultivars in the same fields should be thoroughly studied before allelopathic cultivars are released to farmers.

Sulfonylurea type herbicides have been intensively used in far-east Asia since the late 1980s due to high efficacy against a broad spectrum of paddy weeds even at extremely low dose (20–50g a.i. ha^{-1}). However, intensive and repeated application of this type of herbicide has resulted in several negative effects such as (1) evolving weed resistance (Valverde et al. 2000) (2) residual effects on the following crops and (3) the disappearance of some susceptible weeds such as *Brasenia schreberi* and *Sagittaria aginashi*, which affects weed biodiversity (Itoh 2004). These phenomenon have enforced the employment of weed management technologies other than chemical control methods.

As indicated by Duke (2002) and Khush (1996), an improvement of allelopathic traits in crop cultivars can be an alternative to reduced development of herbicide-resistant weeds due to repeated application of herbicides with a similar mode of action, although the added-allelopathic trait is unlikely to completely replace other weed management technologies.

10.5.2 Approaches in Breeding Allelopathic Rice

From the breeding perspective, the distinction between allelopathy and competition is somewhat difficult in a farmer’s field because interference is the phenomenon that really counts (Courtois and Olofsdotter 1998). In this regard, the best way might be to breed allelopathic cultivars having high competitiveness.

There are two approaches to creating more allelopathic crops: (1) classical breeding methods; and (2) genetic engineering.

10.5.2.1 Classical Breeding Method

If a high number of QTLs with little effect are involved, a traditional breeding method can be a reasonable approach (Courtois and Olofsdotter 1998). The principle of traditional breeding for genetic traits is simple; two parents with contrasting behavior are crossed and recombinant inbred lines (RILs) are derived through the single-seed descent (SSD) method.

This procedure consists of advancing the F_2 without selection for two or three generations (sometime four to five generations) in such a way that each F_4 or F_5 seed traces back to a different F_2 plant. Only one seed is retained from each plant in each generation. This will be repeated for two or more generations and afterwards, plants having desirable characteristics can be selected. Once a reasonable degree of fixation is obtained, the allelopathic potential of the RILs can be evaluated. Then, the seed is increased and tested under field conditions for use as allelopathic cultivars.

Recently, Ma et al. (2006) developed a potent allelopathic cultivar, K21 made from a cross between Donginbyeo (non-allelopathic cultivar, but a high yielding variety with good quality) and Kouketsumochi (an allelopathic cultivar, more similar to a wild type) and its generation was advanced by the SSD breeding method. They identified the F_5 generation of this cross that had a desirable agronomic traits and exhibited allelopathic potential as determined by the water extract method. This was repeated in F_5 and F_6 generations under field conditions. Ma et al. (2006) selected one line (K21) that retained some allelopathic potentials with similar agronomic characteristics to the female parent, Donjinbyeo.

Table 1 shows the allelopathic effects of leaf and root extracts of the three rice cultivars on the growth of barnyardgrass. A newly bred K21 line showed higher inhibitory effects than the female parent, Dongjinbyeo which was a non-allelopathic cultivar, but lower than the male parent, Kouketsumochi which was a allelopathic cultivar. Total phenolic compounds of K21were in between the two parents, showing a similar trend to the inhibitory effects (Kim and Shin 2005). Less is understood about inheritance of phenolic compounds. Figure 3 shows the total amounts of some selected phenolic compounds known as exerting allelopathic effect. Among selected phenolic compounds, p-coumaric acid was present in the highest concentration. This result was similar to our earlier work presented in Fig. 4. The newly bred line showed higher total phenolic content than a non-allelopathic cultivar, but lower content than an allelopatic cultivar, indicating a strong possibility of inheritance of phenolic compounds.

The SSD method is simpler than the pedigree method. The SSD method has several advantages including (1) a quick increase of the additive variance among families, (2) the need for only small breeding space and saving of work, and (3) suitability for low heritable traits where visual selection is not effective (Moreno-Conzales and Cubero 1993).

Another method is to cross two parents with contrasting traits through backcrossing to produce near-isogenic lines (NILs) carrying different genes. The allelopathic potential of the NILs can be determined when a reasonable degree of fixation is obtained. Despite the influence of biotechnology in changing future production methods for breeding new crop varieties, research on traditional plant breeding and breeding methodology will play a significant role in future breeding programs. Further refinement of these methods and better knowledge of classical methods are prerequisite for logical use of new tools such as molecular markers.

Table 1. Inhibitory effect of shoot and root extract of three rice cultivars on the growth of barnyardgrass (cited from Kim and Shin 2005)

Cultivar	Extract	Inhibition of barnyardgrass growth (%)					
		Shoot			Root		
	Conc. (%) plant part	1.0	2.5	5.0	1.0	2.5	5.0
Dongjinbyeo	Leaf	6.3	12.1	23.6	6.0	13.8	40.8
	Root	16.2	17.2	20.0	19.2	24.1	46.8
K21	Leaf	31.2	63.9	64.4	27.6	52.2	76.6
	Root	14.4	22.7	26.2	24.9	36.5	54.3
Kouketsumochi	Leaf	16.8	44.2	64.3	18.1	40.6	96.5
	Root	–7.8	12.9	46.6	–8.1	13.7	11.8

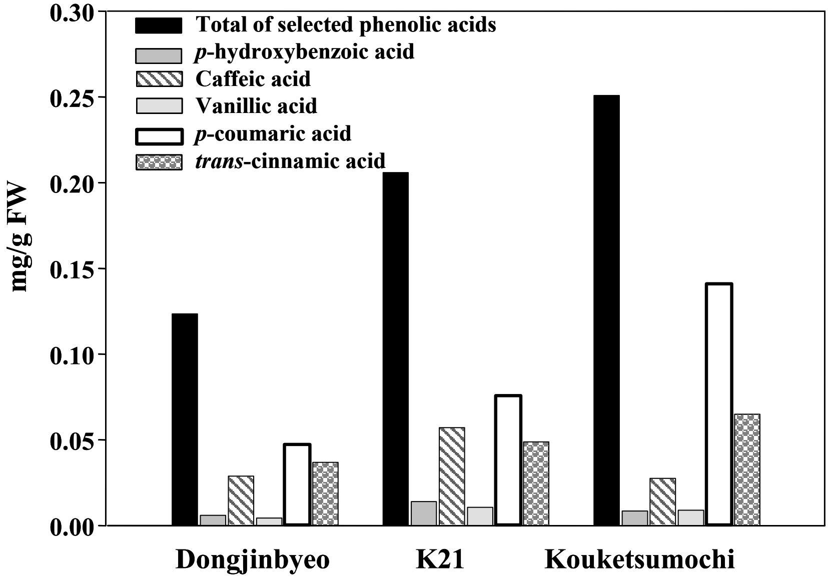


Fig. 3. Total of some selected phenolic compounds of a newly breed rice line, K21 with their parent (cited from Ma et al. 2006)

10.5.2.2 Genetic Manipulation of Rice Allelopathy

Two methods to create more allelopathc crops have been suggested: (1) regulation of gene expression related to allelochemical biosynthesis and (2) direct insertion of genes to produce allelochemicals that are not found in the crop (Duke et al. 2001).

Much of the presently available information on engineering allelochemicals comes to us through efforts to overproduce valuable secondary metabolites in plants (Canel 1999). Relating to the molecular approach for weed control, there is a good reference recently published by Gressel (2002).

Most secondary metabolites being used as allelochemicals are very complex and a multi-gene system might have developed and transformed into the specific crop to produce allelochemicals (Gressel 2002). This seems hypothetical, but opens up new areas for research. Various plant species suppress other species by the production of allelochemicals, which are not toxic to the originating plant but to other vegetation. It is presently known that molecular approaches in breeding allelopathic cultivars are potentially much more complicated than developing a herbicide resistant crop or producing a crop with resistance to insects or pathogens.

10.5.2.3 Regulation of Gene Expression

In order to regulate gene expression, the main requirements are to identify the target allelochemical(s), determine the enzymes and genes encoding them and to insert a specific promoter into crop plants in order to enhance allelochemical production. The enzymatic steps involved in the biosynthesis of the major classes of phenylpropanoid compounds are now well established and many of the corresponding genes have been cloned (Dixon et al. 2002).

The biosynthesis of phenolic compounds is regulated by many enzymes in the phenylpropanoid pathway. It is valuable to identify the phenolic compounds corresponding to enzymes. In our recent work, p-coumaric acid was found to be present in the highest amount of any intermediates of the phenylpropanoid pathway in several allelopathic rice cultivars used. Figure 4 shows the various phenolic compounds detected in different rice cultivars. The concentration of p-coumaric acid was three to five times higher in Tang gan, Kouketsumochi and Taichung Native 1 than any of the other cultivars when quantified from rice leaves harvested just before the heading stage. Based on this result, it can be postulated that the level of p-coumaric acid in plants will be a parameter for determining plant–plant interactions in rice.

Cinnamic acid 4-hydroxylase (CA4H) is the enzyme catalyzing cinnamic acid to p-coumaric acid, which is a key reaction in the biosynthesis of a large number of phenolic compounds in higher plants. The activity of CA4H was measured to elucidate how the activity is influenced by UV irradiation in rice leaves.

CA4H activity in Kouketsumochi was induced by UV and its peak activity was observed at 24 h after UV irradiation for 20 min. AUS 196 showed no response, however, indicating a differential response to UV or other environmental stresses among rice cultivars (Fig. 5). This further indicates that some metabolites having allelopathic potential might be newly synthesized or highly elevated in rice plants by UV irradiation (Kim et al. 2000a). Due to increased content of p-coumaric acid in rice plants irradiated by UV, it is assumed that the *CA4H* gene plays a role in the elevation of the allelopathic function in rice plants.

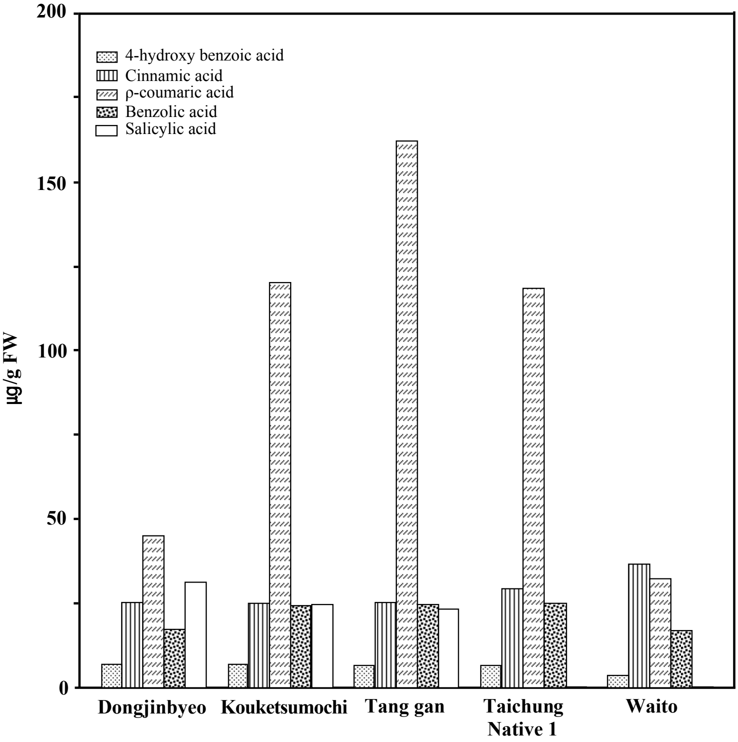


Fig. 4. Intermediate compounds in the phenylpropamoid pathway in various cultivars identified by HPLC. The rice leaves were extracted with 10 mL methanol and 20 uL of extracted sample was injected into a CLC-ODS column. TN1: Taichung Native 1 (cited from Shin et al. 2000)

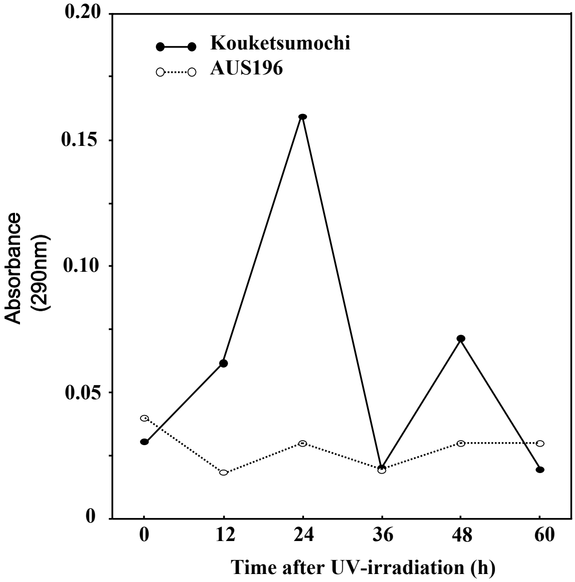


Fig. 5. Change in the CA4H activity of different rice cultivars affected by UV-irradiation. The rice plants were harvested at the indicated time after UV-Irradiation (cited from Shin et al. 2000)

In order to investigate a specific promoter that confers responsiveness to UV light, the constructs of various Capsicum annum sesqiterpene cyclase (CASC) promoters were fused onto a *GUS* gene. The levels of *GUS* activity for transgenic plants with pBI121-KF1 and pBI121-KF6 were significantly elevated by UV-irradiation and had approximately 2- to 3-fold increase over the untreated-transgenic plants. In contrast, *GUS* expression in the transgenic plants with pBI121-CaMV 35S was not changed by UV, and in the other constructs, had only a very small increase (Back et al. 1998). The regulation of genes associated with allelopathy can be achieved by developing a specific promoter responsive to plant-weed competition or environmental stresses (Shin et al. 2000). The specific promoters, the CASC-KF1 and KF6 were fused to *CA4H* gene. The gene constructs were introduced into the binary plant expression vector pIG121-HMR with reverse primer harboring BamHI site and forward primer harboring HindIII site.

10.5.2.4 Insertion of Genes to Produce Allelochemicals

Rice produces momilactone diterpenoids such as phytoalexins and allelochemicals. Wilderman et al. (2004) identified the enzyme, a syn-copalyl diphosphate specific 9β-pimara-7, 15-diene synthase (OsDTS2) catalyzing syn-CPP to be converted to polycyclic hydrocarbon intermediate syn-pimara-7,15-diene which is the precursor of momilactone A and B.

The approach we have been undertaking is to alter existing biochemical pathways by insertion of two genes such as CA4H and OsDTS2 into a cultivated rice variety to produce ρ-coumaric acid and momilactone, respectively and simultaneously in the presence of weeds under field conditions. Studies on development of a rice variety producing herbicidal substances through multiple genes integration have been undertaken for the last several years. This approach seems difficult to employ, but will eventually produce the desirable results we have expected. In this regard, there was a good review paper done by Duke et al. (2001) and a reference book, molecular biology of weed control published by Gressel (2002).

10.6 Application and Prospect

It was noted that more than 100 years of selection and breeding in barley have resulted in a dilution of genes from landraces and consequently a declining allelopathic activity (Bertholdsson 2005). Both competitive ability and allelopathic activity will likely continue to decrease in barley germplasm in the future if no direct selections for these traits are done (Bertholdsson 2005). It is a widespread belief that similar trends might be occurred in rice germplasm.

There is a need for allelopathic traits, especially in organic farming systems, where herbicides can not be used and in areas close to water reservoirs where herbicide use is restricted. Direct seeding is spreading throughout Asia to overcome labor and water shortages, which are key limiting factors for transplanted rice. In direct-seeded rice, weeds and rice emerge together. The early stage weed management capable of suppressing weed growth is becoming more important. Another increasing problem is worldwide development of herbicide resistant weeds, which may force breeders to consider breeding for competitiveness and allelopathy as a high priority area. Modern cultivars that combine early vigor and high specific allelopathic activity should be selected to allow for an effective weed management with reduced herbicide input (Bertholdsson 2005).

To breed allelopathic rice crops, a search for highly allelopathic cultivars or allelopathic traits from rice germplasm should be intensively undertaken. For an improvement in rice allelopathy, we first need more elaborate and universal screening method and identification of allelopathic traits, allelochemicals and genes regulating allelopathic effects. More thorough understanding on genetic control of allelopathy will help breeders incorporate allelopathic traits into future rice crop.

Different categories of compounds have been reported as rice allelochemicals. Thus, there is a possibility that several allelochemicals remain to be identified. It is assumed that multiple genes are involved in the production of some allelochemicals (Einhellig 1995). Therefore, incorporation of two or more specific genes related to rice allelopathy into modern commercial varieties by biotechnology would give us a bright future for the development of allelopathic crops.

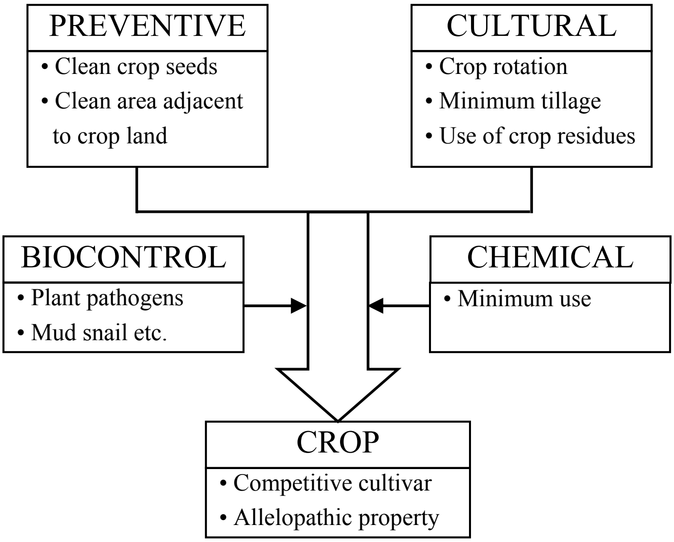


Fig. 6. Proposed components of IWM (modified from Kim 1993)

However, in order for this technology to be practically used in rice production systems, the potential problems such as autotoxicity, metabolic imbalance, residual effect and development of a tolerant population upon repeated culture of such cultivars should be thoroughly investigated before they can be released.

The restoration and enhancement of allelopathic activity will be also made from the conventional breeding methods as Ma et al. (2006) improved allelopathic potential of the commercial rice variety. It takes time, but is possible for transferring allelopathic traits to commercial cultivars by traditional breeding technique, if allelopathic lines or accessions are available.

Rice residue such as straw, rice flour and hulls can be directly used for weed control purpose. Rice straw produces a number of acids when decay, which exhibit inhibitory effect on paddy weeds, and rice flour and hulls have been used for weed management in organic farming. Farmers in Korea generally leave rice hulls in the field after harvesting, although studies show that over 14,850 kg ha^{-1} may be required to have weed inhibitory effect (Jung et al. 2006). Incorporation of the allelopathic plant materials to the paddy field at 1–2 tons ha^{-1} can reduce weed biomass by about 70% and increase yield by about 20% (Xuan et al. 2005).

It is generally known that allelopathy alone may not completely replace the weed management technologies, but it could surely function as a component of integrated weed management (IWM) technology. Figure 6 shows the components, which could be included in a package of IWM under rice production system in temperate conditions. It schematically presents some of the ways in which, preventive method, cultural practices, biocontrol and allelopathy can be integrated with appropriate herbicides to achieve satisfactory control. It is almost certain that IWM will be the most profitable strategy, but its main limitation is its relative complexity compared to herbicide use. The question is how do we simplify the complexity of IWM technology. This will be the important task for weed scientists in years to come.

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11. Rice Allelopathy Research in China

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Abstract. This article reviews advances in the investigation of rice allelopathy with particular reference to China. We first evaluated several bioassay methods commonly used in laboratory bioassay. Then we reviewed the present status of the investigation on quantitative genetics, quantitative trail loci (QTLs) mapping, genetic diversity, and molecular ecological properties of allelopathy in rice. It was apparent that allelopathy was characterized by quantitative inheritance. Several QTLs for allelopathic effect on the target weed plants such as barnyard grass have been determined and mainly located on chromosome 1, 2, 3, 5, 8, 9 and 12, which were significantly mediated by external environment. The increased allelopathic potential in rice exposed to stresses was due to the increased expression of genes involved in synthetic metabolism of phenolic compounds and the decreased expression of enzymes was associated with terpenoid synthetic metabolism.

11.1 Introduction

Rice (*Oryza sativa* L.) is a staple food crop for billions of people worldwide. Weeds are among the most severe and widespread biological constrains to rice production. Their uncontrolled growth in crop field may cause 45~95% loss in yield (Lin et al. 2000a; Moody 1991). This problem has been was traditionally tackled by extensive use of chemical herbicides. However, the increased herbicide resistance in weeds and concerns about harmful environmental effects of herbicides has put pressure on farmers to reduce their use. It is essential to find sustainable and environmentally acceptable alternative weed management methods. Weed control by “allelopathy” has drawn increased attention.

Dilday et al. (1991) first reported on rice allelopathy. Since then, several groups around the world have been working on screening rice cultivars for allelopathic potential in order to evaluate the possibilities for utilizing allelopathic features in rice for weed control. In Chinese literature, there were many ancient records about allelopathic phenomena. Since the last decade of 20th century, allelopathy has been a new focus of research, especially in rice (Lin et al. 2006; He et al. 2005). This chapter summarizes the findings on rice allelopathy research in China, and discusses the current challenges, opportunities and future direction in the research of rice allelopathy.

11. 2 Screening Methods for Evaluation of Rice Allelopathic Potential

A plant with allelopathic potential is referred to as the “donor plant”, while the plant in the vicinity affected by the allelopathic compounds from the donor plant is called the “receiver plant”. In allelopathic interactions, some phytotoxic substances are released by donor plants into the environment to affect the growth of receiver plants. Laboratory bioassay is the first step used to investigate the possible involvement of allelopathy (Foy 1999). Many bioassays have been designed to identify the role of allelopathy in donor–receiver interactions (Pederson 1986; Shilling and Yoshikawa 1987; Weidenhamer et al. 1989; Liu and Lovett 1993; Haugland and Brandsaeter 1996). Many bioassay methods are in use to detect allelopathic potential in rice germplasm and we know that the same rice cultivar may respond differently with different screening bioassays. Ideally bioassays should be conducted with plant species naturally occurring or cultivated in association with presumed allelopathic plants. An appropriate screening bioassay should meet several requirements: convenience and reliability, cost effective, rapidity, and easy to operate, broad application to numerous target species, reproducible and statistically valid, require limited time and space and sensitive to detect the differences of allelopathic activity among accessions.

Keeping these in mind we selected eight different rice cultivars, PI312777, Lemont, Moroberekan, IAC25, IAC47, IAC120, Batatais, Iguape Cateto etc. in the bioassay to test the allelopathic potential of rice on the target weed, barnyard grass (*Echinochloa crusgalli* L.) by using these different bioassay methods, relay seeding in agar (RSA), relay seeding in silica (RSS) and seeding in rice root-exudation (SRE) (Shen et al. 2004).

Table 1. Analysis of variance on inhibition rates of rice cultivars tested with 3 methods*

Variance source	Square sum	Degree of freedom	Average of variance	Fiest value
Block	0.042	2	0.021	1.884
A factor	0.683	2	0.342	30.375**
B factor	1.964	7	0.281	24.952**
A×B	1.060	14	0.076	6.732**
Error	0.517	46	0.011	
Total	4.265	71		

*A factor refers to three different bioassay methods i.e. relay seeding in agar (RSA), relay seeding in silica (RSS) and seeding in rice root-exudation (SRE). B factor refer to eight rice cultivars. **It was significant at 1% level.

We found that RSA method significantly increased the detection of allelopathic potential by 86.4 and 91.3%, compared to that by SRE and RSS, respectively. But no differences were found between SRE and RSS. The eight rice cultivars possessed higher inhibitory rate on the target weed by RSA than that by SRE and RSS. However, their inhibitory rates varied by SRE and RSS. PI312777, IAC47 and IAC120 showed higher inhibition by SRE and RSS. The reverse was true in other accessions, indicating that the same rice cultivar showed marked difference in inhibitory response by SRE and RSS. We concluded that RSA was the best bioassay method by which allelopathic potential of each rice cultivar can be evaluated (Table 1, Table 2).

Table 2. IRs of rice cultivars tested with 3 methods and their significant comparison*

Rice accessions	Inhibitory rate			
	RSA	SRE	RSS	Average
PI312777	57.2	43.2	35.4	45.0abA
Lemont	10.8	–33.4	4.2	–6.0eD
Moroberekan	23.4	14.6	17.6	18.4dC
IAC25	50.0	35.1	45.0	43.6abA
IAC47	45.3	35.9	21.0	34.1bcAB
IAC120	46.9	42.6	17.3	24.4cdBC
Batatais	47.5	13.5	35.8	35.9abAB
Iguape Cateto	58.2	38.2	42.1	46.2aA
Average	44.0aA	23.6bB	23.0bB	30.2

*The data in this table was average of three replications. The small letter indicates significant at the 5% level, whereas the capital letter shows significant at the 1% level.

We successfully used this method (RSA) to evaluate the allelopathic potential of 57 rice accessions with different allelopathic potential on barnyard grass (Table 3). Five rice cultivars such as Iguape Cateto, PI312777, Azucena, Taichung Native 1 and IAC25, showed over 50% inhibitory rate (IR) on barnyard grass root growth. IRs of twelve cultivars, such as AU257, Red Rice5, IAC164 and Mafeng 1 ranged from 40 to 50%. IRs of 21 cultivars including Taizhung 189, Dinorado, Arroz de campos, Shuangzhan 2 ranged from 30 to 40%. IRs of 13 cultivars, such as Fengaizhan, IR721413, IR60080-46A, IR72412 ranged from 20 to 30%, while IRs of six cultivars including Zhengyou 1, IR55423-01, Pratao Precoce, Aisanruzhan, Muxiang 25 and Lemont were less than 20%. The results showed that the number of rice accessions under different allelopathic potentials fit well to normal distribution as showed in Fig. 1, implying that only small percentage of rice germplasm in the collected accessions showed strong allelopathic potential in suppressing the target weeds.

The RSA provided a rapid, simple and inexpensive procedure for screening rice accessions with allelopathic potential against a target weed under laboratory conditions. In this method, the roots of donor plants were distributed throughout the agar medium and fully interacted with receiver plants. Allelochemicals released from the roots of donor plants were diffused into the agar medium to affect the root growth of the receiver plants. The continuous growth of donor plant seedlings ensured a constant release and accumulation of allelochemicals into the agar medium, which was almost similar to the continuous growth in nature.

Table 3. Inhibition rates of fifty-seven rice accessions on the root of barnyard grass by RSA*

No.	Cultivars rice accession	Resource origin	Root length of barnyard grass	Inhibitory rate IR (%)
1	Iguape Cateto	Brazil	18.3±7.8	58.4±1.6
2	PI312777	America	19.0±5.1	56.8±2.3
3	Azucena	The Philippines	20.3±4.6	53.9±1.4
4	Taichung Native 1	Taiwan	21.8±5.2	50.2±8.9
5	IAC25	Brazil	21.9±5.4	50.0±4.7
6	AU257	Bangladesh	22.7±5.1	48.4±0.6
7	Red Rice5	China	22.8±5.6	48.2±6.8
8	Batatais	Brazil	23.1±5.5	47.5±5.2
9	IAC120	Brazil	23.5±7.0	46.7±7.4
10	Co39	India	24.1±8.8	45.2±2.6
11	IAC47	Brazil	24.1±6.2	45.1±7.5
12	IR72417-3R-8-2	The Philippines	24.4±5.5	44.5±13.2
13	Yehuazhan	China	24.8±8.9	43.6±5.9
14	IR70617	The Philippines	25.0±6.5	43.2±6.7
15	Jingyouzhan	China	25.9±6.1	41.1±8.6
16	IAC164	Brazil	25.9±5.4	41.1±6.9
17	Mafeng 1	China	26.0±6.9	40.9±10.1
18	Taizhong 189	China	26.7±7.3	39.3±8.6
19	Dinorado	The Philippines	26.7±7.8	39.3±2.2
20	Vandana	Colombia	27.2±6.3	38.2±5.6
21	IAC165	Brazil	27.5±8.6	37.5±1.1
22	IR56	The Philippines	27.9±5.3	36.6±4.7
23	Shuangmeizhan	China	28.0±9.0	36.4±6.4
24	IR70651	The Philippines	28.3±5.7	35.7±2.4
25	IR36	The Philippines	28.8±6.9	34.5±4.3
26	Dee Geo Woo Gen	Taiwan	28.9±7.0	34.3±3.8
27	Dular	Egypt	29.0±7.7	34.1±3.1
28	IR73382	The Philippines	29.1±8.8	33.9±2.8
29	IR71331	The Philippines	29.3±6.3	33.3±9.4
30	Chaoerzhan	China	29.3±7.3	33.3±6.3
31	Sanyizhaozhan	China	29.6±7.3	32.7±6.7
32	Wab56-125	Ivory Coast	29.7±6.1	32.5±4.1
33	Polha Murcha	Brazil	29.9±10.0	32.0±6.6
34	Qisanzhan	China	30.0±6.9	31.8±7.5
35	Dourado Pecoce	Brazil	30.0±8.2	31.8±3.5
36	Bala	India	30.1±9.5	31.6±6.7
37	Arroz de campos	Cuba	30.5±9.1	30.7±7.9
38	Shuangzhan 2	China	30.6±9.5	30.4±7.5
39	Fengaizhan	China	31.2±7.8	29.1±5.8
40	IR721413	The Philippines	31.3±8.9	28.9±4.7
41	Qidaizhan	China	31.3±6.6	28.9±8.8
42	IR73384	The Philippines	31.8±9.8	27.7±9.4
43	IR64	The Philippines	31.8±9.3	27.7±5.3
44	Xinsimiao	China	32.0±7.0	27.3±1.3
45	Daishuzhan	China	32.1±6.8	27.0±4.3
46	Qingxiangzhan	China	32.2±7.5	26.8±6.1
47	IR62266-42-6-2	The Philippines	32.6±10.2	25.9±4.0
48	IR65907-116-1-B	The Philippines	33.4±9.4	24.1±9.1
49	Moroberekan	Guinea	33.8±8.6	23.2±6.6
50	IR60080-46A	The Philippines	34.6±5.4	21.4±4.1
51	IR72412	The Philippines	35.1±6.1	20.2±7.8
52	Zhengyou 1	China	35.4±7.6	19.5±1.9
53	IR55423-01	The Philippines	35.9±9.4	18.4±2.5
54	Pratao Precoce	Brazil	36.0±8.0	18.2±2.6
55	Aisanruzhan	China	36.2±9.7	17.7±4.6
56	Muxiang 25	China	37.8±9.1	14.1±2.1
57	Lemont	America	39.2±9.3	10.9±3.2
	CK		44.0±10.2	

*CK, Control= barnyard grass (*E. crusgalli* L.) grown alone.

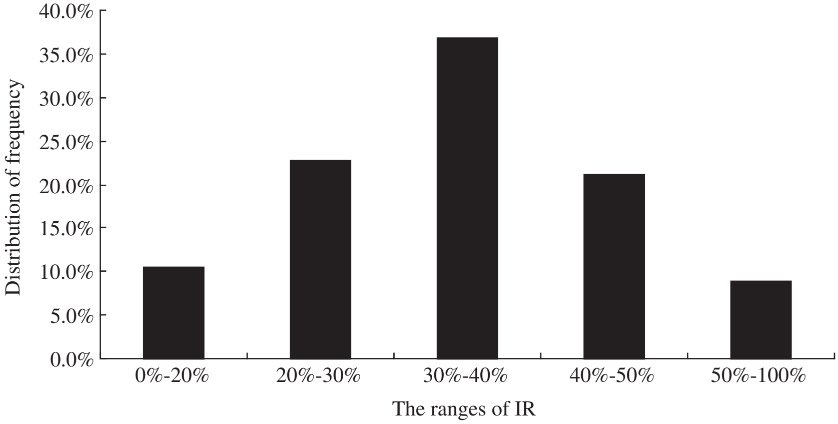


Fig. 1. The distribution of frequency on 57 rice accessions in different intervals of IR values

11.3 Genetic Diversity in Allelopathic Rice

From the above results one could argue that the genetic variability of the allelopathic trait in rice accessions is widespread. Therefore, when a significant level of intra-accession variability in allelopathic expression become evident, it is necessary to assess their genetic diversity (Motiul et al. 2001). However, information on the use of molecular markers for the characterization of genetic diversity in allelopathic germplasm of crops is limited. More recently, we used molecular markers of inter-simple sequence repeats (ISSR) to evaluate their relationships at the species level and to establish their potential usefulness as genetic resources in breeding for allelopathic cultivars resistant to weeds. In this study we tested 57 rice accessions from 10 countries/regions against barnyard grass as receiver plants. Eighty pairs of primers purchased from Shengong Inc. were first screened for PCR. Seven pairs of ISSR primers that generated clear, reproducible banding patterns were chosen for the ISSR analysis of *rice*. A total of 34 bands were scored corresponding to an average of 4.9 bands per primer on the basis of the presence (1) or absence (0) in the bands. The size of the amplified fragments ranged from 200 to 1300 bp. Among the thirty four loci, 18 were polymorphic (53%) at the species level of *rice* (Table 4).

Table 4. The primer sequences and their ISSR band data used for the diversity analysis

Primer	Sequences (5'~3')	Total bands	Polymorphic bands	PPB (%)
7	$(AG)_8T$	7	3	42.9
9	$(AG)_8G$	4	4	100.0
25	$(AC)_8T$	6	4	66.7
34	$(AG)_8CTT$	4	1	25.0
43	$(CT)_4AGA$	4	2	50.0
50	$(GT)_8CTC$	4	1	5.0
73	$(GACA)_4$	5	3	60.0

Further analysis showed that UPGMA clustering method differentiated the 57 rice accessions into six main groups based on the genetic similarity, GS=0.79 (Lin et al. 2005). The first group was composed of 31 cultivars, of which 13 were from Mainland China, nine from the Philippines, five from Brazil, two from America and the others from Guinea and Ivory Coast. In this group, four cultivars showed over 40% inhibition of barnyard grass roots. There were 18 accessions in the second group including six lines from China, five from Philippines, three from Brazil, two from India and the others from Bangladesh and Colombia, of which 11 accessions showed more than 40% inhibition. There were only three accessions clustered into the third group. Iguape Cateto from Brazil and PI312777 from America resulted in 58.4 and 56.8% inhibition respectively, and Arroz de campos from Cuba had a lower IR value (30.7%). There were also three cultivars in the fourth group, two from the Philippines and the other from China, all having lower IRs. IR73384 from the Philippines and Dourado Pecoce from Brazil were clustered into the fifth and sixth groups respectively. IRs of these two accessions was low (27.7–31.8%) as shown in Table 3 and Fig. 1.

As the genetic variability of the allelopathic trait appear to be widespread rice it was essential to investigate the underlying genetic diversity of the accessions. The genetic polymorphism of allelopathic rice accessions detected by ISSR method showed that accessions from the same geographical location could be clustered into one group. Some rice accessions with higher allelopathic potential also could be grouped together, implying that the genes conferring allelopathy in those accessions might be isolocus, such as rice accessions IAC25, IAC47 and IAC120. Also, PI312777 and Taichung Native 1, from America and Taiwan respectively, were clustered together and all possessed high allelopathic potential. This could be supported by the fact that PI312777 was hybrid progeny from Taichung Native 1 and Taichung 65 (Dilday et al. 1998). However, some accessions with different allelopathic abilities were clustered in the same group. Iguape Cateto, Dourado Pecoce and Pratao Precoce exhibited lower levels of genetic polymorphism. The tendency might be attributed to oriented selection for other desirable traits in breeding program. It has been postulated that wild types of existing crops once might have possessed high allelopathic activity and that this character inadvertently attenuated through continuous selection of crop plants for other desirable characteristics (Putnam and Duke 1974).

11. 4 Molecular Mapping of QTLs Associated with Allelopathic Effect on the Target Plants

Based on DNA molecular markers and inhibitory rate (IR), many germplasms with different genetic backgrounds and different allelopathic potential were examined through cluster analysis. The results were useful to provide the basic information to select the pairs of reasonable parents for genetic and breeding studies. It has been shown that rice allelopathy possess quantitative characteristics controlled by multiple genes suggesting that mapping quantitative trait loci (QTLs) should be a useful approach. Once the genes of interest have been located and the molecular markers closely linked to those genes have been found, the identification of QTLs for allelopathic effect in rice would be useful to provide basic information for selection and breeding of rice cultivars with strong allelopathic effects.

Several research groups are studying the QTL mapping associated with rice allelopathy on the target weeds in China. Zeng et al. (2003) located the QTLs controlling allelopathy using a population of 123 Double Haploid lines (DH) derived from a cross between the *Indica* rice cultivar ZYQ8 (strong allelopathy) and *Japonica* rice cultivar JX17 (weak allelopathy). Four QTLs were identified and mapped to chromosomes 3, 9, 10 and 12 by assessing the growth inhibition of water-soluble extracts from rice seedlings on lettuce seedlings (a model plant). They performed 1.65, –1.44, 1.43 and –1.58 additive effects, contributing to 10.24, 8.02, 8.27 and 9.79% of the total variation in allelopathic effect.

Xu et al. (2003) employed a population of 134 recombinant inbred lines (RILs), to evaluate the allelopathic effect of rice on barnyard grass. The RIL population was derived from a cross between the *Indica* rice cultivar Zhong 156 × Gumei 2 with the map containing 168 DNA markers and covering almost all 12 chromosomes with 1447.9 cM spans. One main effect QTL on the 7th chromosome, explaining 32.30% of the phenotypic variation. Six pairs of digenic epistatic loci were also detected and they accounted for 47.83% phenotypic variation in allelopathic activity, showing the digenic epistatic effect higher than the main QTL effect.

The study was also conducted to identify the QTLs controlling the allelopathic effect of rice on lettuce root growth using RILs derived from a cross between a *Japonica* rice cultivar Asominori and an *Indica* rice cultivar IR24 (Dong et al. 2006). A total of three QTLs associated with allelopathic effect on lettuce root growth were identified and located on chromosome 2, 8 and 11, explaining 6.31, 7.14 and 15.34% of the total variation respectively.

More recently, the RILs derived from the cross of rice cultivar Lemont (weak allelopathy) with Dular (strong allelopathy) were applied to map the alleles associated with allelopathy against barnyard grass (Jia 2006; Xiong et al. 2007). Five hundred and eight pairs of SSR markers were used to polymorphism screening. At last, five QTLs associated with allelopathy against the target weed were detected and located on 1, 1, 5, 9 and 9 linkage groups within RM283-RM128, RM128-RM297, RM421-RM26, RM270-RM434 and RM410-RM553 accounting for 23.97, 15.21, 13.89, 18.63 and 6.64% of genetic variation respectively (Table 5).

Table 5. Major QTLs and their effects on allelopathy of rice

QTLs	Chrom	Marker interval	Position	LOD score	Add	Dom	R^2	S
qAP-1a	1	RM283-RM128	66.6100	2.3378	0.0553	0.0000	0.2397	5.4013
qAP-1b	1	RM128-RM297	81.7100	1.9516	0.0464	0.0000	0.1521	0.9720
qAP-5	5	RM421-RM26	117.6100	5.2289	0.0343	0.0000	0.1389	1.3530
qAP-9a	9	RM270-RM434	52.6100	1.9910	0.0392	0.0000	0.1863	0.2708
qAP-9b	9	RM410-RM553	73.1100	2.7193	0.0237	0.0000	0.0664	1.0671

These results showed that allelopathic effect in rice is a complex quantitative trait and the QTLs associated with allelopathic effect differs in rice cultivars. Today, many QTLs for allelopathic effect of rice were detected on all 12 chromosomes as shown in Table 6. The number of observed QTLs associated with main allelopathic effects located on chromosome 1, 3, 5, 9 was the largest, suggesting that many QTLs were correlated with allelopathic effects in rice, and it is possible to make a combination of different allelic QTLs associated with allelopathic effect on the target weeds to produce new cultivars with strong weed inhibition capabilities by using marker assisted selection (MAS) method.

Table 6. The distribution of QTLs controlling allelopathy on 12 chromosomes

Linkage group	1	2	3	4	5	6	7	8	9	10	11	12
The number of QTLs	4	3	4	1	4	1	2	3	4	1	2	3

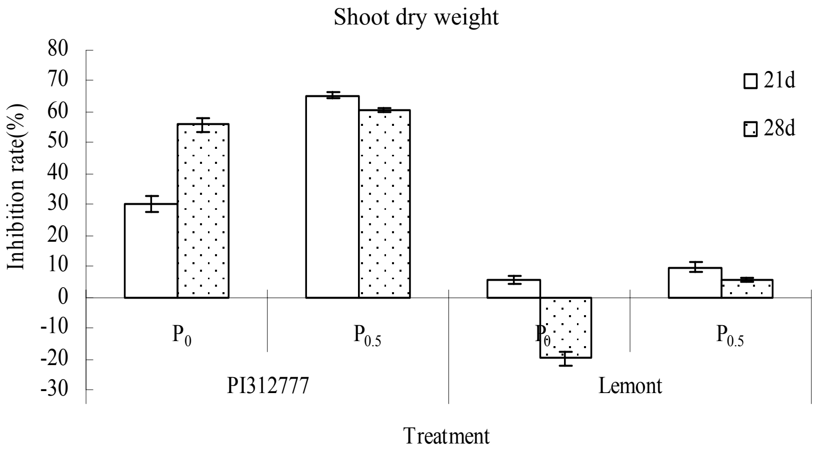
11.5 Properties of Molecular Ecology in Rice Allelopathy Under Stress Conditions

An early observation showed that allelopathic effects might be subjected to other environmental conditions and/or added nutrients. Other studies in the last century demonstrated that phenolic allelopathy might be more severe under low fertility and that raising the nutrient level could suppress some of the allelochemical effects (Waller 1987). Strove and Osborn reported that the toxicity of vanillic and ρ-coumaric acids to barley plants depended closely on nutrient concentrations. Two-way analysis of variance showed a definite interaction between phenolic treatments and nitrogen–phosphorus supply, and that ρ-coumaric acid effects were dependent upon nitrogen levels. At low nutrient levels the phenolic compounds showed significant inhibition effect, suggesting that soil fertility might be critical in phenolic allelopathy. Hall et al. (1982) found that pigweed grown in soil amended with chlorogenic acid was stunted and that the plants phosphorus content had been reduced. However, addition of a nitrogen–phosphorus–potassium supplement negated those effects. Indeed, case studies showed inputs of nitrogen and phosphorus alleviated allelopathic inhibition on goldenrod, tall fescue and sunflower. Several phenolic acids and many nonspecific allelopathic conditions have been shown to alter the mineral content of plants. Certainly, phenolic allelochemicals can interfere with cellular functions in a number of ways that are of importance to plant nutrition. These instances illustrate the importance of interaction between the two stress conditions. The reasons or mechanisms behind these phenomena are still largely unknown. But it is certain that stress exacerbates the effects of some allelopathic compounds. Furthermore, factors such as temperature, water, nutrient limitations and plant density, can all significantly modify plant response to some allelochemicals.

As a result, an increasing number of studies have focused on the relationship between environmental variation and plant allelopathy. Although research on rice allelopathy has made progress, there still remains a significant knowledge gaps on the mechanism of rice allelopathy in the responses to nutrient stress. We tried to explain the physioecological processes and the mechanism of the suppressive effects of rice on barnyard grass under stress by using two cultivars, allelopathic rice PI312777 and non-allelopathic rice Lemont introduced from the U.S.A. as donor plants and barnyard grass as receiver plant. The two rice cultivars each co-cultured with barnyard grass and exposed to low phosphorus condition ($P_{0.5}$, 0.5 mg P_2O_5/L) in the hydroponic culture.

Our results showed that the shoot and root weights of the target weed barnyard grass were significantly inhibited by the co-cultured PI312777 compared to the control (Lemont), regardless of the nutrient condition. The IR varied from 67.06 to 30.17%. Lemont caused less than 10.71% dry weight (DW) reduction in barnyard grass at the 21st day in hydroponic culture, indicating a lesser ability to suppress the growth of barnyard grass than the PI312777. The DW of shoots and roots of barnyard grass co-cultured with PI312777 in the P_0 treatment (normal condition, 10 mg P_2O_5/L) increased slightly 28 days after the treatment. Nonetheless, the shoot and root DWs were significantly reduced by 55.83 and 58.80%, respectively, indicating a high inhibition effect on the target weed. In the case of Lemont, the co-cultured weed was suppressed only under $P_{0.5}$ condition (P-stress) with a slight DW reduction. The result implied that P-stress enhanced the allelopathic effect which is consistent with our previous reports (Lin et al. 2003a). Conversely, the co-cultured weed growth showed slight improvement under normal nutrient condition (P_0) (Fig. 2).

It was also found that PI312777 significantly inhibited the weed's root activity, as shown in Fig. 3. Under P_0 and $P_{0.5}$ nutrient conditions, the average root activity of the weed co-cultured with PI312777 was significantly reduced by 28.77 and 37.02%, respectively. This suggested that PI312777 had a stronger suppressive ability on the weed's root activity than its counterpart. In turn, the weed growth and DW were reduced. The same tendency was found in the case of Lemont co-cultured with barnyard grass exposed to low phosphorus condition. However, much lower IR was found in Lemont than in PI312777. Especially, when treated with P_0, the IR was 2.99% after 21 days causing slight stimulation (6.99%) after 28 days, as compared to PI312777 exposed to the same conditions. This study indicates that PI312777 significantly inhibit N, P and K absorption in the target weed (Fig. 4). The N, P and K in barnyard grass were reduced by 15.04–16.51%, 9.49–22.25%, and 35.14–39.03%, respectively, under P_0 condition after 21–28 days. When exposed to the $P_{0.5}$, the mineral contents in barnyard grass were decreased by 7.71–10.87%, 45.04–45.53%, and 18.60–25.96%, respectively. Lemont also showed a slight inhibitory effect on the contents of N, P and K in the target weed under different phosphorus supplies. Under P_0, N, P and K contents in barnyard grass were reduced by 3.07–6.30%, –3.26–2.05%, and 6.03–8.39%, respectively after 21–28 days in the treatment. In the case of $P_{0.5}$, the contents decline only by 1.61–4.15%, 3.92–5.02%, and 4.17–6.72%, respectively. This indicate that PI312777 suppressed N, P and K absorption of barnyard grass significantly greater than Lemont, regardless of the level of nutrient supply, which was attributed to allelopathic effect on the weed.



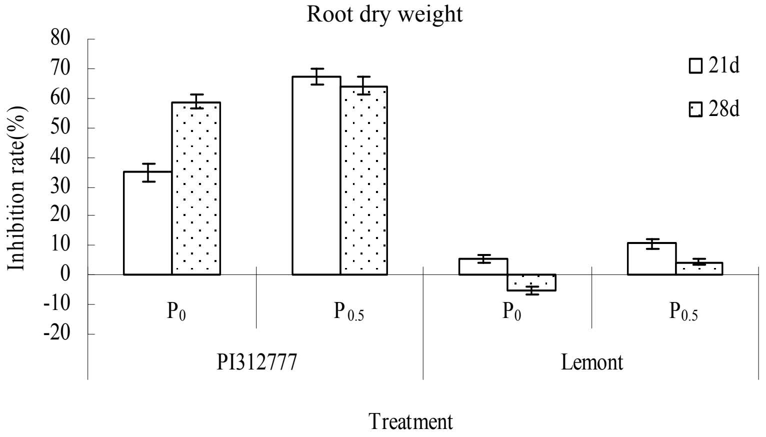


Fig. 2. Comparison of IR on shoot and root dry weight of barnyard grass co-cultured with rice cultivars PI312777 and Lemont under different phosphorus supplies (Source: Shen and Lin 2007)

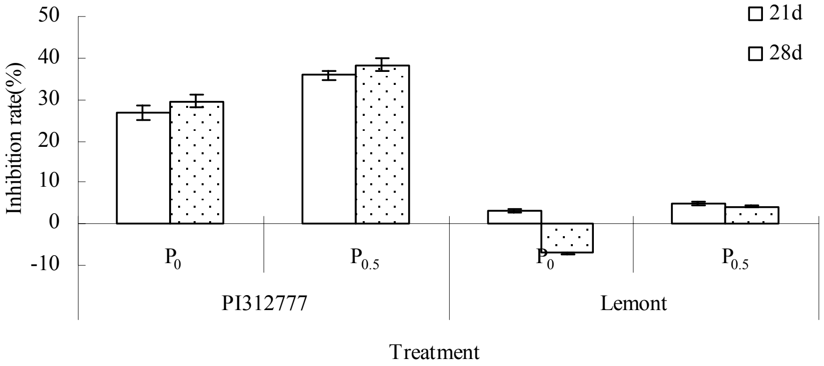


Fig. 3. Comparison of IR on root activity of barnyard grass co-cultured with rice cultivars PI312777 and Lemont under different phosphorus supplies (Source: Shen and Lin 2007)

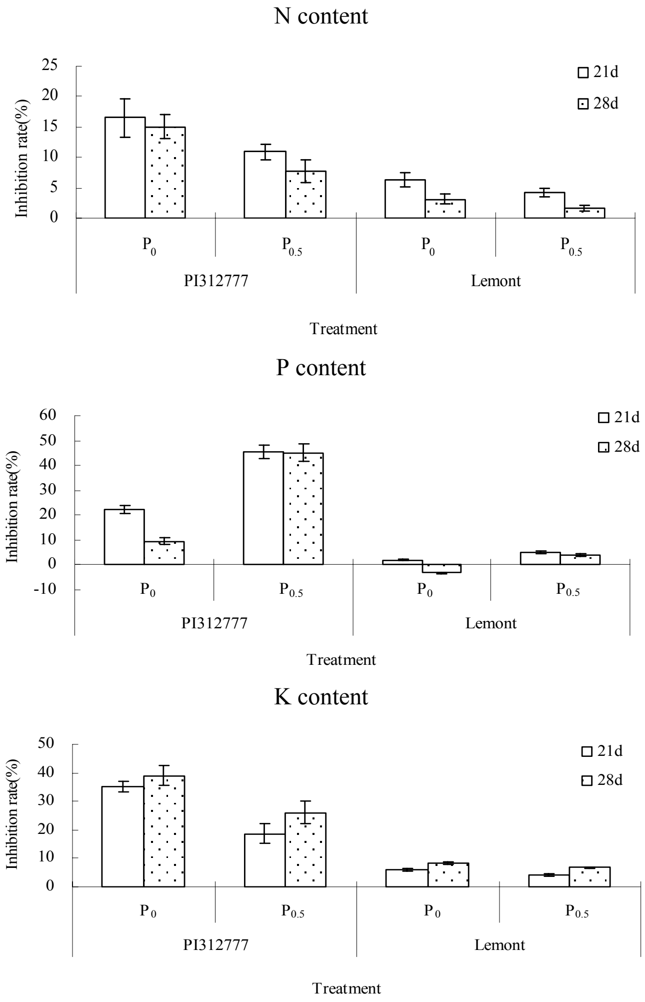


Fig. 4. Comparison of inhibition on N, P and K contents of barnyard grass co-cultured with rice cultivars PI312777 and Lemont under different phosphorus supplies (Source: Shen and Lin 2007)

The two rice accessions showed significantly higher inhibition of P contents in barnyard grass under $P_{0.5}$ than that under P_0, but the opposite was observed on N and K contents the target weed (Fig. 4), indicating that in $P_{0.5}$ (low phosphorus condition), the weed took up more N and K nutrient than that in P_0 (normal condition) because of physiological compensation effect. However, under the same phosphorus condition, N and K absorption of the weed co-cultured with allelopathic rice accession PI312777 was much lower than that in weed co-cultured with the counterpart Lemont, confirming that allelopathy in rice might play an important role in the inhibition on the nutrient source utilization of barnyard grass. This phenomenon might reveal one mechanism that under stress, allelopathy enhanced resource competition but reduced physiological compensation effect. With respect to the physiological process it has been shown the activity of superoxide dismutase (SOD) in barnyard grass was suppressed by the co-cultured PI312777. With the increase of phosphorus stress time, the IR on the enzyme activity of the target plants was significantly enhanced. The reverse was true in the case of Lemont, indicating that when barnyard grass was co-cultured with non-allelopathic rice accession Lemont, a significant stimulation on SOD activity in the target weed, barnyard grass was detected under both P_0 and $P_{0.5}$ conditions in the hydroponic culture. However, the enzyme activity was decreased with increase in stress time (Fig. 5), implying that the barnyard grass co-cultured with non-allelopathic rice Lemont was not suppressed severely and possessed the better physiological status under the nutrient stress.

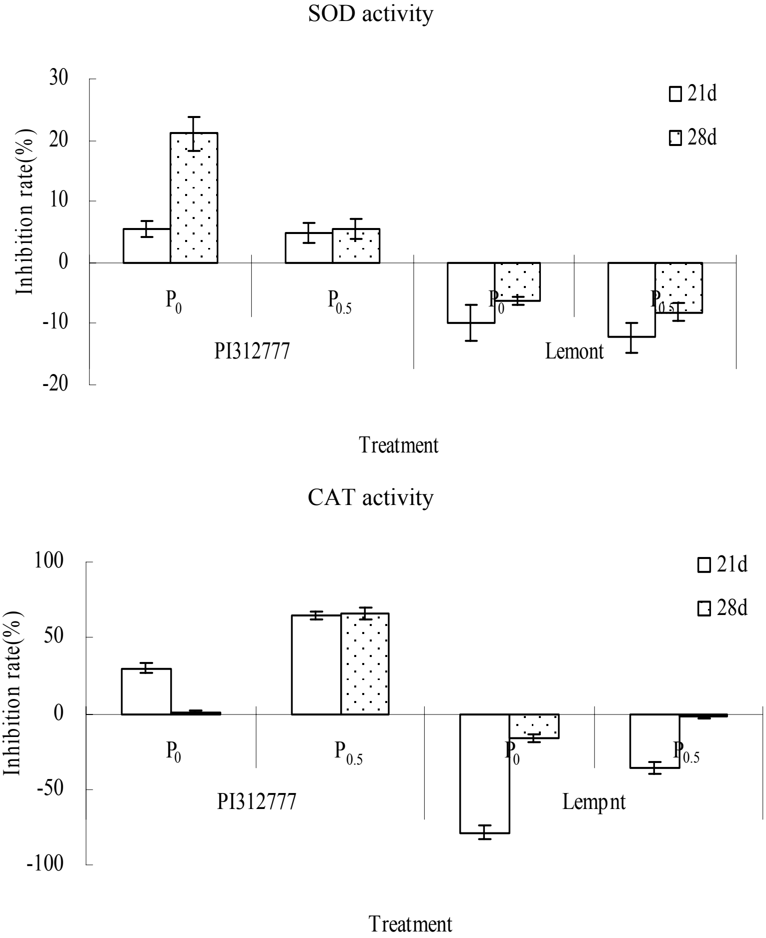


Fig. 5. Comparison of inhibition on SOD and CAT activity of barnyard grass co-cultured with rice cultivars PI312777 and Lemont under different phosphorus supplies (Source: Shen and Lin 2007)

A similar tendency was also found in catalase (CAT) activity of barnyard grass co-cultured with the two rice accessions under different phosphorus supplies (Fig. 5). The results suggested that allelopathy is a very complex phytochemical and molecular ecological process. It is worth to further study this phenomenon under natural condition including resource competition. However, distinguishing the effects of resource competition and allelopathic still remains a formidable challenge (He et al. 2002a) although relay seeding technique distinguished allelopathy from competition in laboratory conditions to some extent (Navarez and Olofsdotter 1996). In this regard the bioassay for separation of allelopathy and competition described by Weidenhamer (1996) made some progress. However, it was effective only when the control was an isogene line of the donor plant (He et al. 2002b). It is time-consuming in breeding for this isogene line. So a new method of allelopathy and competition separation (ACS) was proposed and employed in our lab (Xiong et al. 2005) to effectively assess the allelopathic potential of donor rice without the influence of biointerference raised by resource competition of barnyard grass to demonstrate the mechanism of changes in rice allelopathic potential under different nitrogen supplies in the mixture trial.

This study showed that the DW inhibition of barnyard grass co-cultured with allelopathic rice PI31277 in the mixture varied in the range of 64.9–72.7%. No marked difference was found in different nitrogen supplies. In contrast, DW of barnyard grass co-cultured with non-allelopathic rice, Lemont was strongly affected and the inhibition was in the range of 67.1–12.7%, showing significantly declined tendency as the nitrogen concentration increased. The DW of barnyard grass co-cultured with Lemont was much higher than that with PI31277 in the mixture under different nitrogen supplies especially in lower N supply (5 mg/L) (Fig. 6). This implied that Lemont exhibited lower ability in resource competition under normal or rich nitrogen conditions (10–20 mg/L), but higher ability in lower nitrogen supply (5 mg/L), which was considered to be the result from econiche competition happened in rice–weed system. However, it still remains to be shown how resource competition and allelopathy occur in the co-cultured system.

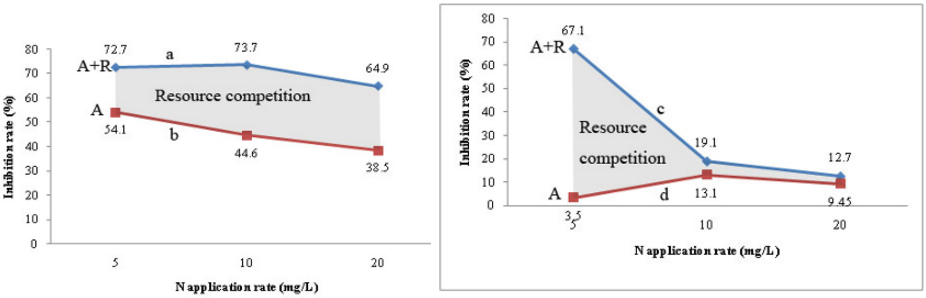


Fig. 6. Deviation curve of biointerference and allelopathy in rice co-cultured with barnyard grass (BYG) refers to the biointerference curve R+A of PI312777 rice accession acting on BYD. b refers to the allelopathic curve A of PI312777 rice accession acting on BYD. c refers to the biointerference curve R+A of Lemont rice accession acting on BYD. d refers to the allelopathic curve A of Lemont rice accession on BYD

Proteomic approach was used to analyze the molecular mechanism for allelopathic effects in rice exposed to the biotic stress of barnyard grass and the abiotic stress of nitrogen supplies (He et al. 2002a; Lin et al. 2001). The four upregulated proteins in the leaves of allelopathic rice accession exposed to the stress of barnyard grass was predicted in the comparison with the control they were 3-hydroxy-3-methylglutaryl-coA reductase 3 (HMGR3), phenylalanine ammonia-lase (PAL), thioredoxin-m and peroxidase precursor shown as spot 3. 4. 1 .2 in 2-DE gels (Table 7). It has been documented that those predicted proteins were mainly involved in the pathway of isoprenoid and phenylpropanoid biosynthesis. The plant 3-hydroxy-3-methylglutaryl-coA reductase (HMGR, EC 1.1.1.34), catalysing the conversion of 3-hydroxy-methylglutaryl-coA (HMG-coA) to mevalonate, the specific precursor of all isoprenoid compounds present in plants, has been considered as a potential rate-limiting enzyme in biosynthesis of phytosterols which originates from cytosolic acetate/mevalonate pathway. The HMGR members that are encoded by multigenes responded differently to various external stimuli including weed and pathogen infection. The induction pattern of HMGR was correlated with terpenoid synthesis. The precise physiological roles of terpenoid have not been defined but it is generally agreed that they mediate plant–environment interactions by playing roles in defense and plant–plant communication. There is evidence that terpenoids have potential use as allelochemicals. Because of their relative low toxicity to vertebrates, they offer significant advantage in weed and pest control application compared with conventional methods (Ha 2003).

Phenylalamine ammonia-lase (PAL) is a key enzyme of plant metabolism catalyzing the first reaction in the phenylpropanoid biosynthesis from L-phenylalanine of a wide variety of natural product based on the phenylpropane skeleton. Phenylpropanoid metabolism comprises a complex series of branching biochemical reactions which provide the plant with a host of important phenolic compounds (Razal 1996; Dixon 2002). It has been assumed that the appearance of phenylpropanoid metabolites during a plant's response to weed and pest infection is a result of the transcriptional activation of the various biosynthetic pathway genes. It has been documented that thioredoxin m and f (Trx-m and Trx-f) link the light-triggered generations of reducing power in thylakoid membranes with the regulation of metabolism in the soluble stroma in higher plant chloroplasts (Duck 2001), photochemically reduced ferredoxin and an iron sulfer protein cleave the unique disulfide bond of Trx which in turn reduces disulfide bonds of chloroplast enzyme. Trx-f is particularly efficient in the stimulation of enzyme involved in the photosynthetic fixation of CO_2, whereas Trx-m functions in the inhibition of one enzyme related to the catabolism of carbon compounds, glucose-6-p-dehydrogenase, which favors hexose monophosphate pathway, HMP, to increase the carbon source for phenylpropanoid synthesis (Dixon 2002; Razal 1996; Lin et al. 2003b).

Table 7. Protein identification of rice by peptide mass fingerprinting analysis

Spot	Access no.	Protein match to	Mw (Da)	pI	Coverage
1	Q9ZP20	Thioredoxin M type, chloroplast precursor (TRX M)	18517	8.16	18
2	P37835	Peroxidase 2 precursor (POD2)	32586	5.51	26
3	Q9XHL5	3 hydroxy 3 methylglutaryl coenzyme A reductase 3 (HMGR3)	59415	7.91	32
4	Q14717	Phenylalanine ammonia lyase (PAL)	75761	8.52	26

It was also found that increased allelopathic potential in rice induced by the stress of lower nitrogen supplies was due to differential expression of proteome involved in the process of signal transduction, stress resistance, allelochemical synthesis, growth regulation (data not shown here). The results obtained from the differential proteomic analysis in our lab showed that allelopathic rice appeared to positively response to the stress of lower nitrogen supplies compared to the counterpart, performing that more upregulated proteins of roots in allelopathic rice accession exposed to lower nitrogen supplies were detected and predicted. It suggest that the enhancement in allelopathic potential of rice exposed to nitrogen stress was related to increased expression abundance of enzymes involved in phenolic synthetic metabolism and decreased expression of enzymes associated with terpenoid synthetic metabolism. These predicted enzymes (upregulated proteins), such as salicylic acid carboxyl methyltransferase 1, cytochrome P450, malonyltransferas, coumarate-CoA ligase and downregulated protein squalene monooxygenase might play very important role in this process, which have been further confirmed in our recent studies by using real-time-PCR (FQ-PCR) (data not shown here) implying that phenolic allelopathy might be the main factor in nutrient stress condition (Wu 2006). The inhibitory effect of allelopathic rice accessions on the target weed seems to have resulted from joint action of many allelochemicals produced in different metabolic pathways.

Kim et al. (2000) reported that the allelopathic effect in rice was increased as the density of barnyard grass co-cultured with the rice was increased. These authors strongly suggest that the rice cultivars were able to increase allelopathic effect when grown under more competitive conditions with barnyard grass. This is in agreement with our previous findings (Lin et al. 2000b, 2003a). We also found that biotic and abiotic stress, such as low nutrient condition and target weed density could stimulate the production of active oxygen species (AOS) and allelochemicals, which in turn induce antioxidant enzyme activities of superoxide dismutase (SOD) and peroxidase (POD) to prevent the accumulation of AOS and increased allelopathic effect in rice exposed to the stress conditions (He 2004; Shen et al. 2004). This physio-biochemical process coupled with the cascades that amplified and transmitted initial signal transduction to activate the relative enzyme gene expression which involved in the pathway of isoterpenoid and phenylpropanoid synthesis. Therefore, it could be inferred that barnyard grass induced differential expression of the proteins in allelopathic rice in the mixed system, and this triggered the pathway related to plant defense (He et al. 2002).

These results suggest that proteomics is an effective tool for physiological and genetic studies in rice allelopathy. It has been documented that allelopathy in rice is quantitatively inherited (Lin et al. 2000a, 2003a). Recently, quantitative trait loci (QTL) technique has been employed to intensively make gene mapping conferring allelopathic traits. However, the gene discovery process is one of elimination and consideration. When a QTL is mapped to an interval of 5–10 cm on a chromosome, there may be 100 genes or more in that interval in which genes become “positional candidate genes” for the QTL. It is difficult to identify the functional genes. Proteomics can contribute to the identification of positional, functional and expressional genes. Comparison of 2-DE protein pattern obtained for key tissue of stressed and control plants will identify a set of stress-responsive proteins encoded by expressional candidate genes. Sequencing of these stressed-responsive proteins will then reveal that some of them have functions clearly consistent with the stress tolerant trait. The encoding genes will be both the expressional and functional candidate genes. This result encouraged us to ascertain the function by transformation and/or reverse genetics by using the functional cloning strategy or RNAi technology.

11.7 Summary and Prospects

Rice being the most important food crop in the world has the possibility to take the lead in this new scientific challenge. The research in allelopathy shows the promising allelopathic activity against weed in sustainable agriculture systems. Bioassays are a necessary tool in allelopathy research, enabling qualification of biological responses in living organisms. The main problem in conducting allelopathy field experiments is that competition and allelopathy cannot be separated in the field. To overcome this problem, various laboratory screening techniques have been developed to measure allelopathy without the interference of competition. Three different bioassays were compared in our laboratory, and the RCA was selected as the preferred one. In germplasm screening, 57 rice accessions have been identified by this method and five of them show indications of strong allelopathic potential. The results obtained from molecular marker analysis showed significant genetic diversity in the rice accessions, implying that it is possible to develop stronger allelopathic cultivars in the suppression of target weeds by recombination and selection in breeding program.

Recent research has shown that the allelopathic potential is a polygenic trait and several QTLs associated with rice allelopathy against the target weed (barnyard grass) were located in different linkage groups (chromosomes), and explained for different contributed rates, ranging from 5 to 25% genetic variation depending on the genetic populations. However, it could be easily affected by environmental factors. Under specific environmental conditions such as biotic or abiotic stress, allelopathic rice accessions are able to enhance the survival interference including their ability for resistance to environmental stress, resource competition and amensalism. The preliminary result indicated that the activity of PAL, the content of phenolic acid and flavonoid in allelopathic rice all increased compared to non-allelopathic rice. The differential proteomic analysis confirmed that the increase in allelopathic potential of rice exposed to stresses is due to increased expression of enzyme genes involved in the synthetic metabolism of phenolic compounds and the decreased expression of enzyme genes associated with terpenoid synthetic metabolism. So isoprenoid and phenylpropanoid metabolisms might be the main pathways to produce allelochemicals in rice.

Several research areas need immediate attention. Large amounts of germplasm must be screened in a search for new allelopathic strains. Efficient procedures must be refined to enable mass screening of a germplasm bank. Recent development in gene microarray, systems biology and the systematic approaches such as genomics, proteomics and metabolomics would useful in revealing the processes and mechanisms involved in molecular ecology of allelopathic rice in the rhizosphere. Close cooperation is necessary among agronomists, ecologists, molecular biologists and plant breeders, as information on existing variability must be incorporated into any sampling design for such a large genepool. The final objective of our research is to design an allelopathic rice cultivar. This design must consider its potentially phytotoxic properties and the ecotoxic features of the allelochemicals from release to degradation; its ecological sustainability; its autotoxicity effect in early growth stages. An integrated multidisciplinary approach is absolutely essential if the challenge of developing a weed-fighting rice cultivar is to be met with success, one that can be used responsibly.

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12. Recent Advances in Wheat Allelopathy

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Abstract. Wheat (*Triticum aestivum*), as one of the world's important crops, has been studied in depth for its allelopathic potential in weed management. Research on wheat allelopathy has progressed rapidly from the initial evaluation of allelopathic potential to the identification of allelochemicals and genetic markers associated with wheat allelopathy. Allelopathic activity varied among wheat accessions. Significant varietal differences in the production of allelochemicals were also found. In comparison with weakly allelopathic accessions, strongly allelopathic accessions produced significantly higher amounts of allelochemicals in the shoots or roots of young seedlings, and also exuded larger amounts of allelochemicals into the growth medium. Genetic markers associated with wheat allelopathy and plant cytochrome P450s encoding the biosynthesis of wheat allelochemicals have been identified. Recent advances in metabolomics, transcriptomics and proteomics will greatly assist in the identification of novel allelopathy genes. Ultimately, the allelopathy genes could be manipulated to regulate the biosynthesis of allelochemicals, thereby resulting in better weed suppression via elevated levels of allelopathic potential in commercial wheat cultivars.

12.1 Introduction

Crop allelopathy has been a particularly intense area of study during the past several decades. Environmental concerns about herbicide use and the occurrence of herbicide-resistant weeds have made crop allelopathy a possible alternative for weed control. A number of crops possess allelopathic potential (Fay and Duke 1977; Nimbal et al. 1996; Wu et al. 2000b; Bertholdsson 2005). Both crop seedling allelopathy and residue allelopathy could be exploited to suppress weeds (Wu et al. 2000a, b). Weed suppression by crop allelopathy at early establishment period could reduce the need for commercial herbicides to early season application, with late season weed control provided by the heightened advantages of crop competitiveness.

Research on the allelopathic potential of wheat (*Triticum aestivum* L.) has advanced rapidly over the last decade. The research has covered a diverse range of areas, including the initial germplasm screening of novel wheat genotypes for enhanced allelopathic potential (Wu et al. 2000b, 2001d; Bertholdsson 2005), identification of allelopathic agents (Wu et al. 2002; Nakano et al. 2006), and the distribution of these compounds within the plant (Wu et al. 2000c; Villagrasa et al.

2006). Research has also progressed to study the phytotoxicity of allelochemicals both individually and collectively (Jia et al. 2006), degradation of these compounds in the soil (Fomsgaard et al. 2004; Macias et al. 2005), and the ecotoxicology of these compounds (Coja et al. 2006). Modern molecular approaches have been employed to study the inheritance of the allelopathic trait, and to identify the genetic markers associated with wheat allelopathy (Wu et al. 2003b).

12.2 Methods for Initial Evaluation of Wheat Allelopathy

Numerous bioassays have been developed to assess the allelopathic potential of a suspected plant species (Macias et al. 2000; Wu et al. 2001e; Belz and Hurle 2004). The development of allelopathic cultivars as a possible strategy for integrated weed management has prompted the search for novel crop genotypes with high allelopathic activity. Concerted efforts have therefore been focused on the design of suitable bioassays for screening large numbers of genetically diverse genotypes and their wild relatives. So far, a number of screening bioassays have been developed to meet different objectives. However, phenotyping of allelopathic activity is extremely difficult due to the confounding effects of competition, especially under field conditions. A specific approach from biological screening (such as extract screening and seedling screening) under laboratory conditions to field testing is proposed so that the requirements for time, space, and labour can be minimized (Wu et al. 2000b).

12.2.1 Aqueous Extract Screening Bioassays

Aqueous extract bioassays have been widely used, due to simplicity, for evaluating residue allelopathy of a suspected donor species. In general, extract bioassays are conducted in petri dishes: Seeds of receiver species are placed on substrata (often filter paper), moistened with aqueous plant extracts of donor species or distilled water as a control (Guenzi et al. 1967; Wu et al. 2003a). The petri dishes are placed in an incubator under light or dark conditions for a given period of time (usually from 2 to 7 days) before germination and radicle elongation are measured.

Aqueous plant extract bioassays have been employed to investigate cultivar differences in the phytotoxicity of wheat (Guenzi et al. 1967; Wu et al. 2003a), and rice (*Oryza sativa* L.) (Ebana et al. 2001). Guenzi et al. (1967) detected differential phytotoxicity of aqueous straw extracts among nine wheat cultivars (*T. aestivum*). In an evaluation of 39 wheat cultivars, Wu et al. (2003a) found that aqueous extracts of different cultivars varied significantly in their allelopathic effects on germination and root growth of a herbicide-susceptible (HS) biotype of annual ryegrass (*Lolium rigidum*); germination was inhibited by 4–100%, and root growth by 19–100%, depending on the cultivar. Similarly, Ebana et al. (2001) reported varying allelopathic activity among one hundred rice accessions by an extract bioassay.

12.2.2 Seedling Screening Bioassays

Young seedling stage of crop plants is a critical growth stage, when an intense competition begins between crop and weed plants. If a crop cultivar can produce and exude potent allelochemicals of sufficient quantities into the soil and suppress weeds in the vicinity during the early seedling establishment period, then crop plants will gain an advantage over the weeds during subsequent periods.

A laboratory screening bioassay, "Equal Compartment Agar Method" (ECAM), was developed to assess wheat seedling allelopathy on annual ryegrass (*Lolium rigidum*) (Wu et al. 2000a). Briefly, pregerminated donor seeds are uniformly selected and aseptically sown on an agar surface in three rows in one half of a glass beaker pre-filled with water agar (nutrient-free). The beaker is wrapped with a piece of parafilm and placed in a controlled growth cabinet. After the growth of wheat seedlings for 7 days, pre-germinated seeds of receiver weed species are then sown on the other half of the agar surface in three rows (Fig. 1). A piece of pre-autoclaved white paperboard is inserted across the centre and down the middle of the beaker with the lower edge of the paperboard kept 1 cm above the agar surface. The entire beaker is thereby divided into two equal compartments that were occupied separately by donor and receiver seedlings. The beaker is again wrapped with parafilm and placed back in the growth cabinet for continuous growth of 10 more days. After 10 days of co-growth of weed and crop seedlings in the growth cabinet, both wheat and weed seedlings are harvested for the measurements of growth parameters.

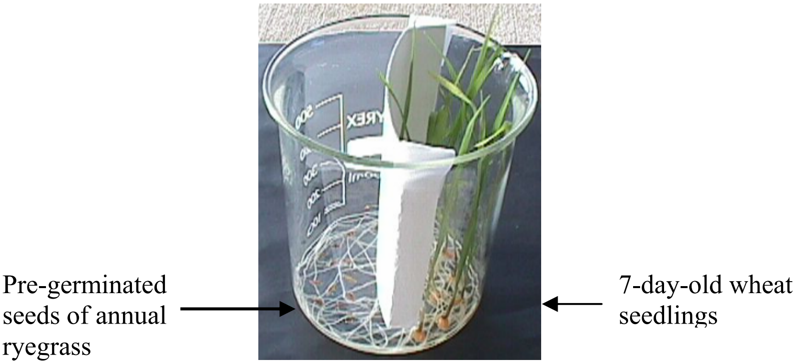

Fig. 1. Pre-germinated annual ryegrass seeds were sown to one side of the beaker with the other side occupied by the 7-day-old wheat seedlings according to the Equal Compartment Agar Method

This bioassay can be further simplified by removing the wheat seedlings after the growth of 7 days, and then sow the target weed seeds. There were no significant differences in the ranking of wheat cultivars with and without the removal of wheat seedlings.

Wu et al. (2000b) used this bioassay to screen a worldwide collection of 453 wheat accessions for novel wheat genotypes with high allelopathic potential. This method was slightly modified to determine the variation in allelopathic activity of root exudates among 1104 wheat accessions from Africa, South America, Asia, the former Soviet Union, America, Sweden and other European countries (Bertholdsson 2004). He found that certain wheat genotypes can produce toxic root exudates, inhibiting the root growth of perennial ryegrass (*Lolium perenne* L.) by as much as 50–60%. An increasing trend in potential allelopathic activity was found in spring wheat during 100 years of breeding, although there is a decreasing trend in barley (Bertholdsson 2005). The high allelopathic activity in some modern spring wheat cultivars could be incorporated into high yielding wheat lines for better weed suppression.

Recently a Dose-Response Bioassay using hydroponic culture was designed to assess the allelopathic activity of 131 accessions of *T. aestivum* L., *T. durum* Dest., and *T. spelta* L. (Belz and Hurle 2001). The bioassay differentiated wheat accessions in their allelopathic activity on the root growth of *Sinapis alba* L. The amounts of hydroxamic acids exuded by different cultivars showed a positive correlation with their growth inhibition in bioassay. This hydroponic bioassay has been described in detail by Belz and Hurle (2004). Briefly, young wheat seedlings with the first leaf through the coleoptile are transplanted into one side of an aluminum-covered glass beaker covered with polyethylene terephthalate lids. Four young receiver plants (*S. alba*) with their cotyledons completely unfolded are then transferred into the other side of the beaker 1 day after the growth of wheat plants in the beaker. Seven plant densities (0–30 plants/beaker) of wheat (the donor) are used in order to calculate ED_{50} (plant density causing ± 50% response) for each wheat accession. Membrane pumps are used to aerate the nutrient solution contained in the beaker. Water losses due to evaporation are adjusted daily. Measurements of root length and weight are undertaken after 6 days of co-growth of the two tested species. Root exudates can be collected for further identification of allelochemicals. The correlation of the cultivar-specific allelopathic potential (ED_{50}) to the allelochemical content in root exudates can be estimated, thereby providing valuable information on the chemical basis for the observed allelopathic inhibition. This bioassay is applicable to all grain crops and to a wide range of receiver species. However, the use of pumps and a range of donor plant densities for each crop accession might limit its adoption in a large scale screening of crop germplasm.

12.2.3 Direct Chemical Screening

The fundamental basis for the expression of crop allelopathy in the field lie in the fact the crop plants are capable of producing allelochemicals and exuding them into the growth environment. Screening the production of these bioactive substances in plants and root exudates should indicate whether or not the tested crop accession possesses high allelopathic potential.

If allelopathic agents are known, direct chemical screening can therefore easily differentiate between allelopathic and non-allelopathic accessions. Following an initial chemical screening, potential accessions are selected and screened further in the greenhouse or the field (Mattice et al. 1999), thereby reducing the amount of time and space required in other screening bioassays. Chemical screening helps to explain the experimental data of laboratory and field bioassays and provides insight in the likely genetic control of the varying allelochemical production between crop varieties (Wu et al. 1999a). Direct chemical screening has been employed to assess the differential production of allelochemicals among crop accessions in oat (*Avena* spp.) (Fay and Duke 1977), barley (*Hordeum* spp.) (Lovett et al. 1994), sorghum (Nimbal et al. 1996), and wheat (Niemeyer 1988; Copaja et al. 1991; Nicol et al. 1992; Wu et al. 2001a).

Intensive research has also been undertaken in determining the differential production of allelochemicals (cyclic hydroxamic acids) in *Triticum* spp. In a screening of 52 Chilean cultivars of *T. aestivum* and *T. durum*, Copaja et al. (1991) demonstrated that cultivars differed in the production of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), ranging from 1.4 to 10.9 mmol/kg fresh weight. Nicol et al. (1992) extended the screening work to a worldwide collection of 47 cultivars of *Triticum* (mainly *T. aestivum*) and found that the contents of DIMBOA ranged from 0.99 to 8.07 mmol/kg fresh weight. Direct screening of 55 accessions of 17 wheat progenitors showed that DIMBOA was highest in *T. speltoides* (16.0 mmol/kg fresh weight) and lowest in *T. tauschii* (Coss.) Schmalh (0.21 mmol/kg fresh weight) (Niemeyer 1988). Recent research in wheat allelopathy also demonstrated the varied levels of DIMBOA and several phenolic compounds among fifty eight wheat accessions in either shoots, roots or in root exudates (Wu et al. 2000d, 2001a, b, c).

Direct chemical screening of a large collection of crop accessions is often limited by the availability of analytical expertise and expensive facilities. It is always important to eliminate the obvious alternate explanations before embarking on months, possibly years, of laboratory chemical work and field tests (Romeo and Weidenhamer 1999). It is essential that chemical screening be combined with other screening bioassays under a controlled environment or in the field.

12.3 Allelopathy of Wheat Residues

The allelopathic influence of wheat residues (straws) in agroecosystems has been well documented (Alsaadawi et al. 1998; Alsaadawi 2001; Zuo et al. 2005a; Li et al. 2005). Wheat straw and its rhizosphere soil have been reported to exhibit inhibitory effects on weeds (Liebl and Worsham 1983; Opoku et al. 1997). Wheat has been successfully used as a cover crop for weed control in various cropping systems (Putnam and Defrank 1983; Blum et al. 2002). Among nine cover crops evaluated, wheat was particularly useful because it was easy to control with chemicals, provided reasonable weed supression, and was least inhibitory to crop seedling establishment (Weston 1990). Blum et al. (2002) reported that the role of wheat root residues appear to be much more important to regulating weed emergence than that of surface shoot residues. Thilsted and Murray (1980) found that the inhibition of *Amaranthus* spp. in wheat straw-mulched plots was approximately equivalent to that obtained with herbicides in straw-mulched and bare-soil plots. Banks and Robinson (1980) also reported that straw mulch suppressed the growth of spiny amaranth (*Amaranthus spinosus* L.), tall morningglory [*Ipomoea purpurea* (L.) Roth], and volunteer wheat more than herbicides used on non-mulched areas. Narwal et al. (1997) reported that wheat residues significantly reduced biomass of broad leaf weeds and grassy weeds in the field.

Aqueous extracts from wheat straw are allelopathic against a broad spectrum of weed species (Steinsiek et al. 1982; Liebl and Worsham 1983; Al-Hamdi et al. 2001). Steinsiek et al. (1982) reported that weed species differed in their responses to the extracts, with *Ipomoea bederacea* and *Abutilon theophrasti* being inhibited the most, and *Echinochloa crus-galli* var. *frumentaceae, Ipomoea Lacunosa* and *Cassia obtudifolia* the least. Al-Hamdi et al. (2001) demonstrated the phytotoxic nature of wheat straw leachate and the possible involvement of organic molecules in the growth inhibition of perennial ryegrass (*Lolium perenne*). These results suggest that allelochemicals in wheat straws might be leached into the soil and influence the growth of certain weeds in the vicinity.

Variation in residue-allelopathy has been reported among wheat cultivars and growing conditions. Higher allelopathic activity was associated with wheat growing under organic than conventional farming systems (Mathiassen et al. 2006). The inhibitory effects were not correlated to the content of DIMBOA and benzoxazolinones in the incorporated wheat residues, indicating the involvement of other allelochemicals. Thirty-eight wheat accessions were evaluated for residue allelopathy against annual ryegrass by an aqueous extract bioassay (Wu et al. 2003a). Aqueous extracts of wheat shoot residues significantly inhibited the germination and root growth of a biotype of annual ryegrass, resistant to herbicides of acetyl CoA carboxylase inhibitors (group A), acetolacate synthase inhibitors (B), photosystem II inhibitors (C), and tubulin formation inhibitors (D). Germination of the herbicide resistant (HR) ryegrass was inhibited from 3% to 100%, depending upon the allelopathic wheat accession. The phytotoxic effects on ryegrass root growth ranged from 12% stimulation to 100% inhibition. These results suggest that residues of certain wheat cultivars with strong allelopathic potential could provide a non-herbicidal alternative for the management of herbicide resistant weeds.

Shilling et al. (1985) claimed that wheat mulches had an allelopathic suppressive effect on some broadleaf weeds. The allelochemicals in wheat residues may kill weeds in the next crop sown into the mulched residues under no-till systems (Worsham 1984).

12.4 Allelopathy of Wheat Seedlings

Wheat has been evaluated for seedling allelopathy on certain weeds. Spruell (1984) screened 286 wheat accessions for allelopathic potential in the United States of America. Root exudates of each accession were compared with those of a commercial strain, T64, for inhibiting root and shoot growth of *Bromus japonicus* and *Chenopodium album*. When one of the allelopathic accessions CI13633 was grown with *B. japonicus* on a one-to-one basis in U-tubes containing aerated Hoagland's solution, growth of the weed was approximately 53% of that recorded when grown with T64.

A worldwide collection of 453 wheat accessions were evaluated for differential seedling allelopathy against *L. rigidum* (Wu et al. 2000b). Results showed that wheat accessions differed significantly in their seedling allelopathy, inhibiting the root growth of ryegrass over a range from 10% to 91%. Of the 453 accessions screened, 63 accessions were strongly allelopathic, inhibiting the root growth of ryegrass by >81%, while 21 accessions were weakly allelopathic, inhibiting *L. rigidum* by <45%.

Wheat allelopathy research has also been extended to wheat progenitors in order to identify novel allelopathic genes for potential use in elite wheat cultivars. Hashem and Adkins (1998) reported that seedling allelopathy differed amongst *Triticum speltoides* accessions on the growth of wild oat (*Avena fatua* L.) and Indian hedge mustard (*Sisymbrium orientate* L.). One out of 17 accessions inhibited root length of wild oat, and two out of 19 accessions inhibited the radicle length of Indian hedge mustard. Based on a bioassay on lettuce, Zuo et al. (2005b) reported that *T. aestivum* was strongly allelopathic, while *Triticum boeoticum*, *Aegilops tauschii* and *Triticum dicoccoides* were weakly allelopathic. *T. monococcum*, *Aegilops speltoides* and *Triticum dicoccum* had moderate allelopathic activity.

12.5 Allelochemicals in Wheat

Advanced analytical techniques such as gas chromatography-mass spectrometry (GC/MS) and tandem spectrometry (GC/MS/MS) have been applied to identify and quantify allelochemicals of plant origin (Wu et al. 1999b; Haig 2001). A number of phytotoxic substances suspected of causing allelopathic effects have been identified in wheat (Neves and Gaspar 1990; Wu et al. 1999b; Nakano et al. 2006). There are three main categories of allelochemicals identified in wheat – phenolic acids, hydroxamic acids and fatty acids.

12.5.1 Phenolic Acids

Phenolic acids have been identified as allelopathic agents in wheat, although the importance of these phenolics as allelochemicals in ecosystems has been questioned because the concentrations needed for phytotoxicity far exceeded the concentrations commonly found in natural conditions (Olofsdotter et al. 2002b). In wheat, *p*-hydroxybenzoic, vanillic, *p*-coumaric, syringic and ferulic acids are most frequently reported, and *trans*-ferulic and *trans*-*p*-coumaric acids are the most predominant acids (Salomonsson et al. 1978; Lodhi et al. 1987; Wu et al. 1999b, 2000c). The phytotoxic effects of phenolic compounds and their mixtures have been demonstrated on a number of weed species, such as *I. lacunosa*, *I. purpurea, Ambrosia artemisiifolia*, *Sida spinosa*, *Trifolium incarnatum* and *Ipomoea hederacea* (Liebl and Worsham 1983; Lodhi et al. 1987; Blum et al. 1992). Bioassays with one of the phenolic acids, *p*-coumaric acid, showed that this compound significantly affected the growth of herbicide resistant (HR) biotype of annual ryegrass (Wu et al. 2003a). *p*-Coumaric acid stimulated the germination of HR ryegrass at the lowest concentration of 0.1 mM, while germination inhibition was found at concentrations greater than 1.0 mM. The root growth of HR ryegrass was significantly inhibited by 22–100%, and shoot growth by 8–84%, depending upon the concentration. The root growth was completely inhibited at concentrations in excess of 5.0 mM. Similarly, five phenolic acids, *p*-hydroxybenzoic, vanillic, coumaric, syringic, and ferulic acids towards *L. rigidum* exhibited little phytotoxicity until their individual concentrations reached a value over to 1 mM (Schulz et al. 1994; Huang et al. 2003).

Wheat accessions differed significantly in the production of phenolic acids. The concentrations of phenolic acids in the 58 accessions ranged from 9.8 to 49.3, 12.9 to 68.8, 0.8 to 11.2, 1.9 to 61.5, 0.2 to 17.0, 11.4 to 117.7, and 3.2 to 149.3 mg/kg dry weight for *p*-hydroxybenzoic, vanillic, *cis*-*p*-coumaric, syringic, *cis*-ferulic, *trans*-*p*-coumaric, and *trans*-ferulic acids in the shoots of 17-day-old wheat seedlings, respectively (Wu et al. 2001a). In the roots, these acids ranged from 24.5 to 94.5 mg/kg for *p*-hydroxybenzoic acid, 19.9 to 91.7 for vanillic acid, 3.7 to 15.4 for *cis*-*p*-coumaric acid, 2.2 to 38.6 for syringic acid, 1.0 to 42.2 for *cis*-ferulic acid, 19.3 to 183.6 for *trans*-*p*-coumaric acid, and 11.7 to 187.6 for *trans*-ferulic acid (Wu et al. 2000d).

Research has also shown that seedlings of different wheat accessions exuded varying amounts of phenolic acids into a growth medium (Wu et al. 2000c, 2001c). Wheat seedling allelopathy was significantly associated with all the compounds analyzed in the shoots, roots, or root exudates. In comparison with weakly allelopathic accessions, strongly allelopathic accessions produced significantly higher amounts of allelochemicals in the shoots and roots of the wheat seedlings, and also exuded larger amounts of allelochemicals into the growth medium (Wu et al. 2000d, 2001a, c, d, 2002).

12.5.2 Hydroxamic Acids

Cyclic hydroxamic acids, a novel class of alkaloids, were identified as another category of biologically active agents conferring weed suppression (Perez 1990; Blum et al. 1992) and resistance to insects and diseases (Niemeyer 1988). Wheat cultivar difference in the production of DIMBOA has also been documented (Niemeyer, 1988; Copaja et al. 1991; Nicol et al. 1992; Wu et al. 2001b; Belz and Hurle 2004). DIMBOA and its decomposition product MBOA (6-methoxybenzoxazolin-2-one) inhibited root growth of wild oats (*A. fatua*) by 50% at concentrations of 0.7 and 0.5

mM, respectively. MBOA also inhibited seed germination of *A. fatua* (Perez, 1990). Blum et al. (1992) found MBOA was more potent than its precursor (DIMBOA) and inhibited germination, radicle and hypocotyl length of *T. incarnatum* and *I. Hederacea*. Huang et al. (2003) reported that two hydroxamic acids 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA), and DIMBOA displayed higher levels of phytotoxicity on *L. rigidum*, quite noticeable at 0.1 mM, while the natural lactam benzoxazinones, 2-hydroxy-1,4-benzoxazin-3-one (HBOA), 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one (HMBOA), required concentrations over 1 mM to exhibit their inhibitory effects. The lower phytotoxicity of HBOA and HMBOA is believed to be associated with the absence of an important OH group on position 4 of the oxazinone ring (Huang et al. 2003).

Fifty-eight wheat accessions were analyzed for the differential production of DIMBOA from the shoots, roots, and root exudates of 17-day-old wheat seedlings (Wu et al. 2001b). DIMBOA content differed significantly in the shoots or the roots between accessions. The variation of the DIMBOA concentration in the 58 accessions was similar for both shoots and roots, ranging from no detectable amount to 730 mg/kg dry matter. Forty-seven out of the 58 accessions did not exude detectable amounts of DIMBOA through their living roots into a growth medium, although substantial levels of DIMBOA were found in the shoot or root tissues. Only 11 accessions were capable of exuding varied amounts of DIMBOA into the growth medium; these amounts ranged between 8.6 and 79.1 μg/L of water agar. These results demonstrated that the exudation of DIMBOA by living wheat roots was highly accession-dependent, indicating that genetic factors govern the exudation process of DIMBOA.

12.5.3 Short and Long-chain Fatty Acids

Fatty acids (aliphatic acids) are claimed as a third category of compounds implicated in wheat allelopathy. The production of short-chain fatty acids, resulting from anaerobic fermentation of the insoluble polysaccharides which represent the major constituents of wheat straw, can also adversely affect crop development in soils of low redox potential (Lynch 1978; Tang and Waiss 1978). Lynch et al. (1980) reported that the acetic acid concentration of freshly harvested straw measured before absorption of soil moisture was 384 mM. Hairston et al. (1987) found that leachate from wheat straw at the concentration of 20 g/L contained 10 mg/kg of acetic acid. Lynch (1977) commented that acetic acid inhibited barley root extension at concentrations of about 5 mM at pH 3.5 and 15mM at pH 6.5. Tang and Waiss (1978) also found that extracts from fermented suspensions of wheat straw inhibited wheat seedling growth. The major allelopathic agents were identified as salts of acetic, propionic and butyric acids. Traces of isobutyric, pentanoic, and isopentanoic acids were also identified (Tang and Waiss 1978; Lynch et al. 1980). Synergistic effects were reported for the combined activities of acetic, propionic, butyric and valeric acids (Wallace and Whitehand 1980). Lynch et al. (1980) concluded that the presence of acetic acid might be a major cause of poor establishment and growth when seeds and seedling roots come into contact with wheat straw.

Propionic acid, one of the fatty acids identified in wheat residues, significantly inhibited the germination and growth of both HR- and HS-biotypes of annual ryegrass. The root growth of both biotypes was significantly inhibited at concentrations over 1.0 mM in comparison with a water control. Complete suppression was found at concentrations greater than 5.0 mM. In comparison with *p*-coumaric acid, propionic acid was more inhibitory to seed germination, shoot growth, and root growth of both ryegrass biotypes (Wu et al. 2003a).

Long-chained carboxylic acids, such as oleic, linoleic and stearic acids have also been identified from wheat (Dong et al. 2005). These fatty acids inhibited the germination of *Leptochloa chinensis*, but had no effects on root growth of the weed.

12.5.4 Other Allelopathic Agents

Allelochemicals in wheat belong to far more than the three categories of compounds mentioned above. As the research progresses, more biologically active compounds are identified. Naphthoic acid, azelaic acid, 1,2,3,5-tetrabromobenzene, and steroidal constituents have been reported in wheat straws (Neves and Gaspar 1990; Gaspar and Neves 1993). They further identified 62 compounds in two allelopathic fractions from wheat straw (Gaspar and Neves 1995). The compounds were carboxylic acid methyl esters, phenolic acids and triterpenoids. The involvement of a cluster of allelochemicals suggests that further research is necessary to understand the chemical basis for wheat allelopathic effects.

Nakano et al. (2006) identified syringoylglycerol 9-O-b-D-glucopyranoside and L-tryptophan from the leachate of the wheat straw. The content of syringoylglycerol 9-O-b-D-glucopyranoside and L-tryptophan in the leachate of wheat straw (100 g eq./l) was 18.5 and 6.2 μM, respectively. Both compounds were inhibitory to the roots growth of lettuce and cress, with L-tryptophan being more potent than syringoylglycerol 9-O-b-D-glucopyranoside.

12.6 Allelochemicals Dynamics in Wheat

The production and exudation of allelochemicals by young wheat plants is a dynamic process. Limited studies have investigated the production dynamics of benzoxazinones (Copaja et al. 1999; Zuniga et al. 1990; Nicol et al. 1992; Nakagawa et al. 1995). Huang et al. (2003) extended the study to simultaneously monitor the exudation dynamics of a range of allelochemicals of two distinct chemical groups, including five benzoxazinones and seven phenolic acids, in the root exudates of young wheat seedlings. Based on the ECAM method (Wu et al. 2000a), wheat seedlings were allowed to grow for 2, 4, 6, 8, 11, and 15 days, respectively. The seedlings were carefully removed from the agar and pre-germinated seeds of annual ryegrass were then aseptically sown onto each of the respective agar surfaces. Ryegrass sown on agar-water with nil-wheat beforehand was used as a control. Ryegrass-containing beakers were allowed to grow for 7 days prior to the measurements of the lengths of ryegrass roots and shoots. They found that all benzoxazinones in wheat root exudates reached maximal concentrations in the day-6 to day-8 zone before declining. Two hydroxamic acids DIBOA and DIMBOA exuded by wheat roots reached their highest levels on day 6 (0.146 and 0.241 µmol/L water agar, respectively), while HBOA reached its highest level (0.424 µmol/L) on day-8. A similar exudation pattern was identified for the total concentration of seven phenolic acids analyzed. The highest combined concentration of measured allelochemicals from both chemical groups occurred at day-8 with a value of 2.3 µmol/L. The exudation dynamics of these allelochemicals agreed well with the observed allelopathic activity on annual ryegrass. Growth inhibition of annual ryegrass by the wheat root exudates in the agar medium increased gradually as the duration of wheat growth in the agar increased, peaked at day 6 and day 8, and then decreased. This allelopathic maximum coincides with the maximization of the total concentration of measured allelochemicals. Such growth inhibition correlates significantly with the levels of the eight allelochemicals (Huang et al. 2003).

12.7 Genetic Control of Allelopathic Activity in Wheat

Although the development of crop cultivars with strong allelopathic potential has not yet been achieved, progress has been made in understanding the genetic control of crop allelopathy. Evidence suggests that allelopathic activity is a heritable trait (Panchuk and Prutenskaya 1973; Dilday et al. 1998). Complex biochemical pathways and distinct categories of allelopathic compounds indicate multiple genes are probably involved in the production of allelochemicals (Olofsdotter et al. 1995; Dilday et al. 1998; Wu et al. 2000b).

Research has revealed that allelopathic activity is quantitatively inherited in wheat, as well as in rice (Dilday et al. 1998; Wu et al. 2000b, 2003b). In a study of allelopathic activity of a population of 453 wheat accessions on annual ryegrass, Wu et al. (2000b) found that wheat allelopathic activity was normally distributed, indicating that this weed-suppressing ability is a quantitative trait. The genetic control of allelopathic activity was further studied using near isogenic wheat lines (NILs) derived from Hartog (weakly allelopathic) × Janz (strongly allelopathic). The allelopathic activity of BC_2-Hartog lines (backcrossed to Hartog) was weak, similar to that of Hartog. Janz lines had strong allelopathic activity, similar to that of Janz. These results suggest that there is a strong genetic basis in conferring allelopathic activity. Niemeyer and Jerez (1997) investigated the chromosomal location of genes controlling the production of hydroxamic acids in wheat using euploid and anueploid Chinese Spring wheat (high in DIMBOA) and substitution lines derived from a cross between Chinese Spring and the variety Cheyenne wheat (low in DIMBOA). It was found that chromosome 4A and 4B may contain genes for the transformation of DIBOA into DIMBOA and chromosome 5B for the transformation of methoxylated lactam into DIMBOA. In addition, there might be a gene in chromosome 4D inhibiting the accumulation of hydroxamic acids.

Recently, molecular techniques have been used to investigate the genetic markers associated with allelopathic activity in wheat (Wu et al. 2003b) and in rice (Jensen et al. 2001; Ebana et al. 2001). A doubled haploid (DH) population of wheat derived from cv. Sunco (weakly allelopathic) and cv. Tasman (strongly allelopathic) was developed to investigate the genetic control of wheat allelopathy (Wu et al. 2003b). Analysis of restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), and microsatellite (SSRs) makers identified two major QTLs on chromosome 2B associated with wheat allelopathy. Regression based on simple interval mapping revealed that one of the QLTs on chromosome 2B accounted for 29% of the total phenotypic variance of wheat seedling allelopathy. Zuo et al. (2005b) claimed that wheat allelopathic activity increased with evolution history, as the genome changed from 2n to 4n to 6n, with a increasing trend of DD < AA < RR < AABB < BB < AABBDD. The results support that genome BB might contain the allelopathic genes.

Jensen et al. (2001) used RFLP analysis to investigate associations between genetic markers and rice allelopathic activity on barnyardgrass in a population of 144 recombinant inbred lines derived from a cross between IAC 165 (*japonica* upland variety, strongly allelopathic) and CO 39 (*indica* irrigated variety, weakly allelopathic). Of the 140 genetic markers examined, four QTLs were identified on three chromosomes, which collectively explained 35% of the total phenotypic variation of the allelopathic activity in the population. In a similar study with RFLP analysis, Ebana et al. (2001) identified seven QTLs on six chromosomes associated with rice allelopathy.

12.8 Genes Encoding the Biosynthesis of Wheat Allelochemicals

The growing interest in plant cytochrome P450s has led to the identification of many plant P450s encoding the biosynthesis of defense compounds, including the notable benzoxazinones (Bxs) in maize and wheat. The biosynthetic pathway of Bxs branches off from that of tryptophan at indole-3-glycerol phosphate. Five genes involved in the downstream reactions from indole-3-glycerol phosphate were isolated from maize and designated as *Bx*1–*Bx*5 (Frey et al. 1997). Bx1 encodes indole synthase homologous to the tryptophan synthase alpha subunit (TSA), whereas *Bx*2–*Bx*5 genes encode four cytochrome P450 monooxygenases (CYP71C1–CYP71C4) (Frey et al. 1997). Expression of cDNAs in yeast demonstrated that each of these cloned P450s catalyzed sequential hydroxylation of indole to DIBOA.

Further study has demonstrated that two genes *Bx6* and *Bx7* are involved in the conversion of from DIBOA to DIMBOA (Frey et al. 2003). *Bx6* encodes a 2-oxoglutarate-dependent dioxygenase that catalyses the hydroxylation of DIBOA at position 7. The resulting product (TRIBOA) is then methylated by the O-methyltransferase *Bx*7 to generate DIMBOA. Two glucosyltransferase genes *Bx8* and *Bx*9 are both able to catalyse the conversion of DIBOA and DIMBOA to DIBOAGlc and DIMBOAGlc, respectively (Rad et al. 2001). *Bx*s genes (*Bx*1–8), governing the entire DIMBOA biosynthesis, have been shown to cluster on the short arm of chromosome 4, although *Bx*9 was located on chromosome 1 (Frey et al. 2003; Rad et al. 2001).

In wheat, cDNAs of five P450s (CYP71C6, CYP71C7v2, CYP71C8v2, CYP71C9v1 and CYP71C9v2) involved in DIBOA biosynthesis were also isolated (Nomura et al. 2002). CYP71C9v1 and CYP71C9v2 shared 97% similarity in amino acid and nucleotide sequences. The cloned P450 species showed 76–79% identity at the amino acid level to the corresponding maize P450 species CYP71C1–C4, suggesting the common origin of DIBOA biosynthesis in wheat and maize. Nomura et al. (2003) further found that these cDNAs (TaBx1–TaBx5) genes were separately located on two groups of chromosomes in wheat. TaBx1 and TaBx2 co-existed in specific regions of chromosomes 4AS, 4BL and 4DL. TaBx3 genes were located on 5AS, 5BS, 5DS and 5BL, while TaBx4 and TaBx5 genes were located on the short arms of group-5 chromosomes.

Since the cloning of the first plant P450 gene in 1990, there has been an explosion in the rate at which genes encoding plant P450s have been identified. Application of modern molecular techniques will rapidly assist in identifying new P450 families and subfamilies encoding biosynthesis of plant secondary metabolites such as allelochemicals. Overexpression of these P450 genes may modify the flux through biosynthetic pathways that give rise to accumulation of allelochemicals. Alternatively, the introduction of foreign P450 genes into alternative host plants may allow for the engineering of novel biochemical pathways and the synthesis of potent allelochemicals for weed suppression.

12.9 Prospects

The genetic enhancement of crop allelopathy provides a novel approach for integrated weed management (Wu et al. 1999a; Duke et al. 2001; Olofsdotter et al. 2002a). However the commercial release of allelopathic crop cultivars will largely depend on our knowledge of allelopathy genetics.

Molecular and biochemical approaches are now being rapidly applied to allelopathy research. DNA microarray technology has been used extensively in gene expression profiling, and in the identification and genotyping of polymorphisms (Aharoni and Vorst 2001). This technology allows the simultaneous detection of the expression of thousands of genes. On the other hand, metabolomics, the unbiased identification and quantitation of all the metabolites, has emerged as a viable counterpart to proteomics and transcriptomics (Weckwerth 2003; Goodacre et al. 2004). Compared to a few target compound analyses, analysis of entire metabolome from root exudates and leaf materials of a suspected allelopathic plant offer greater potential to uncover the chemical basis of allelopathy and improve our understanding of chemical interactions between plants.

Multi-parallel analysis of genomics, transcriptomics, metabolomics, and bioinformatics provides exciting opportunities to discover novel structural and regulatory genes in the biochemical pathways of specific plant metabolites, such as alkaloids, flavonoids, and isoprenoids (Ohlrogge and Benning 2000; Forkmann and Martens 2001).

With the completion of rice and *Arabidopsis* genome projects, enormous DNA and gene sequence data are available from various genome databases. As a result, a candidate gene approach will help identify genes responsible for potent defense compounds in wheat. Transcriptomics and metabolomics analyses of existing wheat genetic stocks would provide comprehensive knowledge on genes or P450s conferring the biosynthesis of natural defense compounds and their chromosomal locations. These alien elite genes will ultimately be introgressed into wheat via chromosome engineering for improved management of weeds.

Once the allelopathic genes conferring the allelopathic activity have been identified, these genes could be cloned and incorporated into modern commercial cultivars to enhance their allelopathic activity for weed suppression. Duke et al. (2001) proposed two strategies for producing allelopathic crops by biotechnology: (1) enhancement of existing allelopathic potential and (2) insertion of genes to produce allelochemicals that are not found in the crop. Further incorporation of allelopathic traits together with crop competitiveness (e.g., early vigour, leaf size, plant height and tillering) (Lemerle et al. 1996) into commercial cultivars could be a major step towards further development of sustainable crop production systems with less reliance on herbicides.

Although effective weed control should not rely solely on the use of allelopathic crop cultivars, the adoption of such cultivars should have a long-term impact on weed management (Wu et al. 2003a). The constant exposure of weed plants to the continuously released allelochemicals even at low concentrations would create a chemically-stressed condition for the weed, thereby altering the normal weed life cycle, such as poor growth vigor, reduction in weed seed production and recruitment in to the soil seedbank. A small change in the reproductive capacity (seed production) will significantly influence the size of seedbank and weed population dynamics in the field (Gonzalez-Andujar and Fernandez-Quintanilla 2004).

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13. Sorghum Allelopathy for Weed Management in Wheat

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Abstract. Weeds cause substantial decline in agricultural production. To overcome weed infestation modern agricultural practices adopted heavy use of a large variety of herbicides. With rising human health and ecological concerns about the adverse effects of indiscriminate use of farm chemicals research on alternative weed management methods is underway worldwide. Exploitation of allelopathic potential of different crop/plant species for weed management under field conditions is one such approach. Sorghum has been reported to contain several allelochemicals in its aerial as well as underground parts. It offers a great promise as a tool for weed management. We conducted a series of field experiments to test allelopathic effects of this crop on weed control and yield of wheat. We found that 10% w/v water leachate of aerial parts of sorghum (also called sorgaab) applied at 30 and 60 days after sowing can reduce weed biomass by as much as 49% with concomitant increase of wheat yield over 20% compared to control. Furthermore, use of sorgaab in combination of herbicides can significantly reduce the amount of herbicide use (by 50%) and get comparable grain yield of wheat as obtained by using the recommended dose of the herbicides. We concluded that sorgaab used alone or in combination with herbicide has a great promise in increasing weed control and grain yield of wheat. Application of this method of weed management has enormous economic and environmental benefits in wheat cultivation.

13.1 Introduction

Although allelopathy is a relatively new field of study, there exists convincing evidence that allelopathic interactions between plants play a crucial role in both natural and manipulated ecosystems. In agro-ecosystems crops, weeds, trees and microbes constitute the biotic component, which not only interact among them but also with the abiotic component of the environment. These interactions are undoubtedly important factors in determining species distribution and abundance in an ecosystem. Logical use of allelopathic effects have a great potential in improving crop production, genetic diversity, maintaining ecosystem stability, nutrient conservation, and above all management of weeds and pests (Altieri and Doll 1978; Putnam and Duke 1978; Leather 1983; Purvis 1990; Einhellig 1996; Swanton and Murphy 1996; Weston 1996; Kohli et al. 1998; Anaya 1999; Chou 1999; Singh et al. 2001).

Weeds are a major threat to agricultural systems causing productivity decline. Modern agricultural practices use large amounts of chemicals to combat weeds and other pests. But the adverse effect of the agrochemicals on the environment including food safety and human heath has prompted urgent need to search for alternative weed management methods. Allelopathy offers a great potential in this direction. However, research on the exploitation of allelopathy of different crop/plant species for weed management under filed conditions has been rather inadequate.

We conducted a series of experiments to exploit allelopathic potentials of a number of plant/crop species for weed control in field crops (Cheema and Khaliq 2000; Cheema et al. 1997, 2000a–c, 2001, 2002a,b, 2003a–e, 2004, 2005; Khaliq et al. 1999). This chapter summarizes our work with particular reference to the use of sorghum allelopathy for weed management in wheat.

13.2 Sorghum Allelopathy

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop grown throughout the world. Because of its rapid growth and ability to suppress weeds it is often chosen as a summer cover crop (Forney and Foy 1985). Allelopathic potential of sorghum has been reported in many studies (Einhellig and Souza 1992; Guenzi et al. 1967; Guenzi and McCalla 1966; Netzley and Butler 1986; Nimbal et al. 1996; Rimando et al. 2001; Weston 1996; Weston et al. 1989; Cheema et al. 2004; Cheema and Khaliq 2000). Spring planted sorghum residues provide up to 90% reduction in weed biomass for 6–8 weeks in no-till summer-planted soybeans (Weston and Czarnota 2001). When sorghum was amended as a green manure, weed biomass in the succeeding alfalfa crops was found to be significantly suppressed (Forney and Foy 1985). Grain sorghum showed inhibitory effects on surrounding weed growth occurring through the following growing season (Einhellig and Rasmussen 1989). Putnam and DeFrank (1983) found that residues of sorghum reduce the number and biomass of common purslane (*Portulaca oleracea* L.) and smooth crabgrass (*Digitaria ischaemum* [Schr.] Muhl.) in the fields by 70 and 98% respectively. They also found that 6–8 week-old sorghum plants suppress the weeds without damaging large seeded legumes and it was noted that younger (2 to 4-week old) herbage (meaning all the aboveground components of the plants) was more effective than older herbage (6–8 week old). Woodhead (1981) reported that young sorghum seedlings were highly allelopathic, and then the effect starts to decline at the age of 28 days followed by reaching the maximum level at heading stage of the plant. The increased allelopathic inhibitory activity of sorghum at maturity was also reported by Cheema and Ahmed (1992). All parts of sorghum plants such as roots, leaves and stems as well as germinating seeds release phyto-inhibitors that can affect growth of grass and broadleaf species such as green foxtail (*Setaria viridis* (L.) Beauv.), velvetleaf (*Abutilon theophrasti* Medik.) and smooth pigweed (*Amaranthus hybridus* L.) (Panasiuk et al.1986; Hoffman et al. 1996). Panasiuk et al. (1986) reported that inter-planting of sorghum reduce the dry weight of barnyard grass [*Echinochloa crus-galli* (L.) Beauv.], redroot pigweed (*Amaranthus retroflexus* L.) and red sorrel (*Rumex acetosella* L.).

Sorghum residues release sorgoleone, cyanogenic glycosides-dhurrin, and a number of breakdown products of phenolics that bring about weed suppression (Guenzi and McCalla 1966; Nicollier et al. 1983; Putnam 1988; Weston et al. 1989;

Weston 1996). Cheema and Khaliq (2000) performed a series of experiments in semiarid region of Punjab, Pakistan, to explore the use of allelopathic properties of sorghum for weed control in irrigated wheat. They observed that spray of water extracts of mature plants obtained after soaking in water for 24 h (also called sorgaab, the term ‘sorgaab’ is derived from two words, ‘sorg’ coming from sorghum and ‘aab’ meaning water in Urdu language) reduced the weed density and biomass of wheat by 35–49% and increased the crop yield by 10–21% compared to control. They further demonstrated that mature sorghum herbage when added to soil at 2–6 Mg ha^{-1} reduce the weeds by 40–50% and increase wheat yield by 15%. The authors concluded that sorgaab could be used as a natural weed inhibitor in irrigated wheat.

13.3 Use of Crop Residue for Weed Management

It has been observed that certain crop residues when used as mulch can exert adequate weed control in subsequent cropping (Liebl et al. 1992; Masiunas et al. 1995; Barker and Bhowmik 2001). Generally speaking, the crop residues effect on weed suppression declines after 4–6 weeks due to the loss of residue mass and breakdown of allelochemicals (Patrick et al. 1963; Kimber 1973; Smeda and Weller 1996). The management of weeds by crop residues is particularly effective under tropical and warm temperate countries because (a) they keep fallow, (b) their cover crops are winter hardy and (c) minimum problem associated with the lowering of temperature by the mulch. Several studies indicate that small seeded crops and weeds are more susceptible to allelochemicals under field conditions than the large seeded plants. This is attributed to greater surface to volume ratio, resulting in more exposure of such seeds to allelochemicals. Furthermore, allelochemicals released by crop residues remain in the upper surface of soil where small seeds are present compared with larger seeds that are sown deeply (Liebman and Mohler 2001). Since the allelopathic effects of crop residues are short lived which also vary greatly with climate and edaphic conditions, these pose a major challenge for weed management.

13.3.1 Use of Sorghum as Crop Residue for Weed Management in Wheat

Incorporation of sorghum (*in situ*) as a whole or various parts of the plant alone or in combination was found to suppress weed growth in wheat (Cheema and Ahmed 1992). The effects of sorghum mulch on weeds were mostly suppressive with the exception of *Melilotus parviflora* which was promoted. Maximum suppression (55–94%) was achieved in *Cyperus rotundus* (Table 1). Allelopathic effects of sorghum depend on the growth stage of sorghum (pre-flowering vs mature plant), the quantity of sorghum biomass added into the soil and the cropping conditions of wheat (irrigated vs non-irrigated). The response of weeds differed in the presence of wheat crop than its absence, which may be attributed to competitive effects of wheat on weeds or its stimulatory effects on *Melilotus* (Table 2). Incorporation of sorghum plant components in soil suppressed broad leaved and grassy weeds including

Table 1. Effect of different components of sorghum (at pre-flowering and mature stage) incorporated in soils of irrigated and non-irrigated wheat fields on *Cyperus rotundus* density

Treatments	*Cyperus rotundus* density (m^{-2})							
	Without wheat				With wheat			
	Irrigated sorghum (1986)	% decrease	Non-irrigated sorghum (1987)	% decrease	Irrigated sorghum (1986)	% decrease	Non-irrigated sorghum (1987)	% decrease
Control (no sorghum added)	147.3 a[1]	–	111.0 a	–	51.0 b	–	67.7 a	–
Pre-flowering sorghum stem and leaf addition	50.7 b	66	30.7 b	72	16.7 e	67	18.3 c	73
Mature sorghum uprooting then wheat planting	35.0 c	76	5.7 e	95	24.3 d	52	25.3 b	63
Mature sorghum roots addition	49.7 b	66	19.0 cd	83	65.0 a	28	5.7 e	92
Mature sorghum root + stem addition	36.3 c	75	9.3 de	92	30.7 b	40	14.3 cd	79
Mature sorghum root + leaf addition	39.3 c	73	13.3 cde	88	39.7 c	22	9.3 de	86
Mature sorghum root + stem + leaf addition	27.0 d	82	8.7 e	92	28.3 d	45	3.7 e	95

[1] Means with different letters in a column differ significantly (DMR test $P \leq 0.05$); % decrease is calculated by comparing with the control (data from Cheema and Ahmed 1992).

Table 2. Effect of different components of sorghum (at pre-flowering and mature stage) incorporated in soils of irrigated and non-irrigated wheat fields on dry weight of weeds (g m^{-2})

Treatments	Without wheat				With wheat			
	Irrigated sorghum		Non-irrigated sorghum		Irrigated sorghum		Non-irrigated sorghum	
	Melilotus[§]	Other weeds[*]	Melilotus	Other weeds	Melilotus	Other weeds	Melilotus	Other weeds
Control (no sorghum added)	7.5 f[1]	116.1 a	8.7 d	120.6 a	18.1 e	23.7 a	9.3 e	26.6 a
Pre-flowering sorghum stem and leaf addition	66.4 b	70.9 c	11.7 d	82.5 b	75.6 a	24.8 a	6.9 e	11.3 d
Mature sorghum uprooting then wheat planting	49.4 c	51.0 d	38.5 a	63.3 c	37.4 cd	16.6 bc	32.1 a	24.8 ab
Mature sorghum roots addition	79.9 a	85.3 b	23.9 c	61.5 c	60.9 b	12.4 d	31.2 ab	15.2 cd
Mature sorghum root + stem addition	23.2 e	33.0 e	23.2 c	41.6 d	41.6 d	19.0 b	16.3 d	14.3 cd
Mature sorghum root + leaf addition	30.3 d	55.8 d	28.0 bc	63.2 c	30.3 d	15.2 cd	33.2 a	13.5 cd
Mature sorghum root + stem + leaf addition	50.7 c	33.7 e	36.8 ab	39.9 d	62.8 b	12.1 d	25.5 bc	10.2 d

[1] Means with different letters in a column differ significantly (DMR test P≤ 0.05); [§] *Melilotus parviflora*; [*] *Rumex dentatus*, *Anagallis arvensis*, *Cyperus rotundus, Phalaris minor*, *Polygonum bellardii*, *Chenopodium album and Senebiera didyma* (data from Cheema and Ahmed 1992).

Cyperus rotundus, Anagallis arvensis, Chenopodium album, Phalaris minor, Rumex dentatus and *Senebiera didyma*. Cheema and Ahmed (1992) reported that soil incorporation of pre-flowering aerial parts of sorghum and roots of mature sorghum increased wheat grain yield by 6 and 12% respectively while soil incorporation of only roots and whole plants of mature sorghum caused 10 and 7% decline in grain yield of wheat (Table 3). Significant yield decline occurred when the crop suffered water shortage. Wheat yields were substantially declined by soil incorporation of mature components of sorghum (Table 3). Soil incorporation of mature sorghum stalks caused significant decline in weed density and biomass with concomitant increase in grain yield of wheat. However, the yield increases were not significant (Table 4). Incorporation of sorghum roots (commonly practiced by the farmers in Punjab) reduced weed dry weight by 21% (Ahmad et al. 1991). Sorghum residues suppressed the density and biomass of weeds as *Rumex dentatus, Phalaris minor, Convolvulus arvensis, Coronopus didymus, Chenopodium album* and *Anagallis arvensis*. However, sorghum promoted *Melilotus parviflora* and *Medicago hispida* density and growth. Effect of sorghum on wheat yield (without fertilizer) was significantly suppressive, while the application of fertilizer mitigated the adverse effects of sorghum on wheat. In the fertilized plots uprooting of sorghum at maturity increased wheat yield by 3.5% while incorporation of any of the plant parts decreased wheat yield (Ahmad et al. 1991). Incorporation of sorghum roots or whole sorghum plant significantly reduced the total and individual weed biomass, except *M. parviflora* and *M. hispidia* which were increased (Ahmad et al. 1991). The effect of sorghum incorporation on weed biomass varied depending on the amount of sorghum biomass and fertilizer added. In wheat crop, soil incorporation of sorghum stalks at 2, 4 and 6 Mg ha^{-1} gave weed mortality of 20, 29 and 41%, respectively (Cheema and Khaliq 2000). The corresponding increases in wheat grain yield were 6, 16 and 17% respectively over control.

Table 3. Effect of different components of sorghum (at pre-flowering and mature stage) incorporated in soils of irrigated wheat field on wheat grain yield (Mg ha^{-1})

Treatments	1986	% ±	1987	% ±
Control (no sorghum added)	2.9 b[1]	–	1.2 b	–
Pre-flowering sorghum stem + leaf addition	3.1 ab	6	1.3 a	12
Mature sorghum uprooting then wheat planting	2.7 c	11	1.2 ab	7
Mature sorghum roots addition	3.3 a	12	1.2 ab	5
Mature sorghum root + stem addition	2.7 c	10	0.9 c	23
Mature sorghum root + leaf addition	3.0 b	1	0.9 c	25
Mature sorghum root + stem + leaf addition	2.7 c	7	0.8 c	30

[1] Means with different letters in a column differ significantly (DMR test P≤ 0.05); % decreases/increases are compared with control. Severe water shortage due to canal closure led to low yield during 1987 (data from Cheema and Ahmed 1992).

Table 4. Effect of sorghum stalk incorporation on weed density and dry weight and wheat yield

Treatments	Weed density m^{-2}	% decrease	Weed dry weight ($g\ m^{-2}$)	% decrease	Wheat grain yield ($Mg\ ha^{-1}$)	% increase
Control	63.7 a[1]	–	19.6 a	–	2.9 b	–
Mature sorghum stem addition at 2 Mg ha^{-1}	50.9 b	20.2	11.3 bcd	(42.0)	3.1 ab	(6)
Mature sorghum stem addition at 4 Mg ha^{-1}	45.0 c	(29.2)	10.0 cde	(48.0)	3.4 ab	(16)
Mature sorghum stem addition at 6 Mg ha^{-1}	37.7 d	(40.8)	8.6 e	(56.0)	3.4 ab	(17)
LSD (0.05)	4.9		2.12		0.6	

[1] Means with different letters in a column differ significantly (0.05 level); % decrease/increase is calculated by comparing with the control; soil incorp.: soil incorporated; major weed flora of experimental field: *Fumaria indica,* Hauskn, *Phalaris minor, Rumax dentatus* L., and *Chenopodium album* L. (data from Cheema and Khaliq 2000).

13.3.2 Sorghum Water Extract (Sorgaab) for Weed Management in Wheat

Several concentrations of sorgaab applied as single and multiple foliar sprays at different DAS showed various levels of weed control and grain yield of wheat (Cheema et al. 1997; Cheema and Khaliq 2000). As shown in Table 5, the highest increase in grain yield (21%) was obtained by using 1:10 w/v ratio of sorgaab applied twice at 30 and 60 DAS with concomitant decreases in weed density and dry weights by 44 and 49% respectively. However, there was no significant difference in increase in grain yield and weed density among the single, double and triple application of sorgaab (Table 5). Therefore, it was concluded that one application of sorgaab at 1:10 w/v ratio should be the recommended dose. Sorgaab sprayed at 10 and 50% concentration suppressed the density of weed species such as *Rumex dentatus, Coronopus didymus, Chenopodium album* and *Fumaria perviflora*. The highest weed density and biomass reduction (36 and 53% respectably) were obtained by using 10% sorgaab applied at 60 DAS with corresponding yield increase of 14% in wheat compared to control (3.39 t ha^{-1}), once again supporting the previous recommended rate of sorgaab use (Cheema et al. 1997).

Cheema et al. (2002a) demonstrated that foliar application of sorgaab (one and two sprays) significantly suppressed the density of weed species such as *Chenopodium album*, *Phalaris minor*, *Avena fatua*, *Convolvulus arvensis* and *Rumex dentatus* from 22–39% depending on the weed species except *Melilotus parviflora* whose density and biomass were either significantly increased or remained unchanged following sorgaab application. From another experiment, Cheema et al.

(2002b) reported that a single spray of sorgaab applied at 30 DAS resulted in 13.5% more grain yield of wheat over control while two sprays of sorgaab enhanced the grain yield by 18.6% over control. They attributed the increase in grain yield to higher leaf area, more fertile tillers, longer spikes, more grains per spike and heavier grains suggesting that the grain yield contributing parameters were promoted either because of suppressive allelopathic effects of sorgaab on weed density and biomass that resulted in higher plant height and straw production which in turn, helped in higher photosynthetic rate and greater translocation of photosynthates to the grains ultimately leading to higher grain yield.

Table 5. Effect of frequency and concentration (dry w/v) of sorgaab (water leachate of sorghum) aerial spray on density and dry weight of weeds, and grain yield of wheat

Treatments	Weed density (m^{-2} at 90 DAS)	% decrease	Weed dry weight (g m^{-2} at 90 DAS)	% decrease	Wheat grain yield (Mg ha^{-1})	% increase
Control	18.1 a[1]	–	34.0 a	–	2.7 e	–
Sorgaab (1:10) one spray 30 DAS	9.8 cd	46	21.3 c	37	3.1 bcd	14
Sorgaab (1:10) two sprays 30 and 60 DAS	10.1 c	44	17.4 d	49	3.3 abc	21
Sorgaab (1:10) three sprays 30, 60 and 90 DAS	9.8 cd	46	17.2 d	49	3.3 ab	22
Sorgaab (1:20) one spray 30 DAS	11.4 b	37	25.5 b	25	3.0 cde	9
Sorgaab (1:20) two sprays 30 and 60 DAS	11.2 b	39	21.7 c	36	3.1 bcd	14
Sorgaab (1:20) three sprays 30, 60 and 90 DAS	10.2 c	44	21.7 c	36	3.1 bcd	15
LSD (0.05)	4.1		2.1		0.3	

[1] Means with different letters in a column differ significantly (0.05 level); % decrease/increase is compared to control (data from Cheema and Khaliq 2000).

13.3.3 Combined Effects of Sorgaab and Fertilizer

Sorgaab application enhanced the positive effects of fertilizer in increasing weed control and grain yield of wheat. Two sprays of sorgaab at the rate of 1:10 w/v applied at 20 and 40 DAS in combination with 114-84-62 kg ha^{-1} NPK gave the maximum weed control and yield increase in wheat (Fig. 1). Similar response of increased weed control and grain yield of wheat was obtained with the application of water leachates of sunflower and eucalyptus in combination with fertilizer (Cheema et al. 2003f).

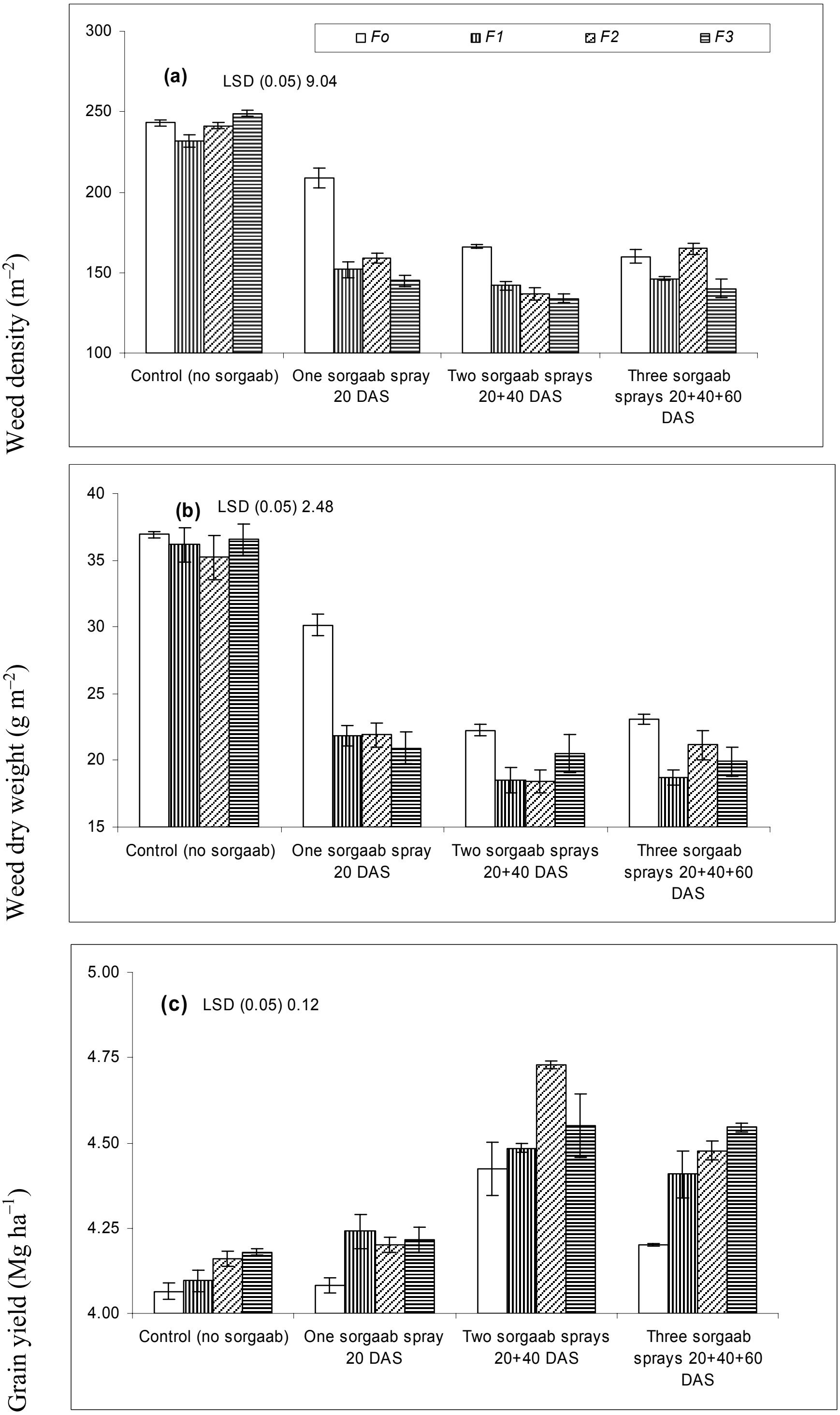

Fig. 1. Effect of sorgaab on (**a**) weed density, (**b**) weed dry weight and (**c**) wheat grain yield under different fertility levels; F0=no fertilizer, F1=84-57-62 kg ha^{-1}, F2=114-84-62 kg ha^{-1}, F3=143-114-84 kg ha^{-1} of NPK (data from Cheema et al. 2002b)

13.3.4 Combined Effect of Sorgaab and Herbicide

Research conducted in our laboratory suggests that there is a tremendous scope for reducing herbicide if it is used in combination with sorgaab (Cheema et al. 2002a, 2003b). For example, the recommended dose of isoproturon, for weed control in wheat could be reduced by 50–60% if it is used in combination with sorgaab at 12 L ha^{-1} (Cheema et al. 2003e). In similar studies for weed control in maize and cotton, they found that half of the recommended dose of atrazine (150 g a.i. ha^{-1}) in combination with sorgaab at 12 L ha^{-1} applied at sowing gave as effective weed control as with the full dose of the herbicide (Cheema et al. 2003a–f). These authors also observed that 1/3rd dose of pendimethalin in combination with concentrated sorgaab at 12 L ha^{-1} gave higher seed cotton yield (72.2%) than the full dose of pendimethalin although weed control was relatively less. A two-year field study was conducted to investigate the feasibility of reducing isoproturon dose in combination with sorgaab for weed control in wheat (Cheema et al. 2003e). Weed suppression during 2001 was the same in treatment combinations of sorgaab at 12 L ha^{-1} + isoproturon at 400, 600, 700 and 800 g a.i. ha^{-1} and full dose of isoproturon at 1 kg a.i. ha^{-1} except sorgaab at 12 L ha^{-1} + isoproturon at 500 and 900 g a.i. ha^{-1}. Two foliar sprays of sorgaab at 12 L ha^{-1} at 30 and 40 DAS suppressed weed density by 32 and 29%, during 2001 and 2002, respectively. Maximum reduction in weed dry weight was obtained with sorgaab at 12 L ha^{-1} + isoproturon at 900 g a.i. ha^{-1} during 2001 and sorgaab at 12 L ha^{-1} + isoproturon at 600 g a.i. ha^{-1} during 2002 (Table 6). These values did not differ significantly from those obtained by using full dose of isoproturon at 1 kg a.i. ha^{-1}.

The treatment combination of sorgaab at 12 L ha^{-1} + isoproturon at 400 g a.i. ha^{-1} was also equal to the full dose of isoproturon at 1 kg a.i. ha^{-1} during 2001 but during 2002, it was less effective with only 57% weed reduction.

Sharif et al. (2005) reported that mesosulfuron methyl at 10.8 g a.i. ha^{-1}, and 1/2 dose of isoproturon at 500 g a.i. ha^{-1} + sorgaab at 12 L ha^{-1} at 30 DAS were most effective by causing 57–85 and 73–81% reduction in total weed density (Table 6). Half of the recommended dose of isoproturon at 500 g a.i. ha^{-1} + sorgaab at 12 L ha^{-1} at 30 DAS was statistically at par in reducing total weed density as with full dose of isoproturon at 1 kg a.i. ha^{-1} at 30 DAS. Similarly, full dose of bromoxinil + MCPA and its reduced dose combined with sorgaab at 12 L ha^{-1} were statistically at par in reducing total weed density. These results indicate that herbicide dose can be decreased by at least 50% when used in mixture with sorgaab at 12 L ha^{-1}. Mesosulfuron methyl at 10.8 g a.i ha^{-1} was the most effective treatment in reducing total weed dry weight by 86% recorded at 60 DAS (Table 6). This was due to better suppression of both annual narrow and broad leaved weeds. It was followed by mesosulfuron methyl at 6.25 g a.i. ha^{-1} + sorgaab at 12 L ha^{-1}, 1/2 dose of isoproturon + sorgaab at 12 L ha^{-1}, bromoxinil + MCPA at 375 g a.i. ha^{-1} and isoproturon at 1000 g a.i. ha^{-1}, and these were statistically at par with one another in reducing total weed dry weight at 60 DAS. Wheat grain yield was significantly increased in all the treatments as compared to control in the range of 5–20%. The maximum grain yield (4.203 Mg ha^{-1}) was obtained in mesosulfuron methyl at 10.8 g a.i. ha^{-1}, and it was statistically at par with isoproturon at 1 kg a.i. ha^{-1}, mesosulfuron methyl at 6.25 g a.i. ha^{-1} + sorgaab at 12 L ha^{-1}, and half dose of isoproturon + sorgaab at 12 L ha^{-1} in increasing grain yield of wheat.

Table 6. Effect of different herbicides (a.i. ha^{-1}) alone and in combination with sorgaab (water leachate of sorghum, L ha^{-1}) applied at 30 DAS on weed density, weed dry weight and grain yield of wheat

Treatments	Weed density (m^{-2})	% decrease	Weed dry weight (g m^{-2})	% decrease	Grain yield (Mg ha^{-1})	% increase
Control	45.1 a[1]	–	4.4 a	–	3.5 d	–
Mesosulfuron methyl (Atlantis 3WG) 10.8 g	6.9 g	85	0.6 d	87	4.2 a	20
Bromoxinil + MCPA (Buctril super 60EC) 375 g	10.6 de	76	1.1 c	75	3.9 b	1
Bromoxinil + MCPA (Buctril M 40EC) 500 g	12.6 c	72	2.3 b	47	3.9 b	9
Isopruturon (Tolkan 50W) 1000 g	9.6 ef	79	1.3 bc	71	4.1 a	18
Mesosulfuron methyl (Atlantis 3WG) 6.25 g + sorgaab 12 L	10.9 cde	76	0.9 c	80	4.2 a	19
Bromoxinil + MCPA (Buctril super 60EC) 215 g +sorgaab 12 L	11.6 cd	74	3.9 a	12	3.8 b	8
Isopruturon (Tolkan 50W) 500 g + sorgaab 12 L	8.8 f	81	1.2 c	76	4.1 a	18
Bromoxinil + MCPA (Buctril M 40EC) 250 g +sorgaab 12 L	17.6 b	61	3.7 a	17	3.7 c	5
LSD (0.05)	1.88		0.39		0.2	

[1] Means with different letters differ significantly at 0.05; DAS: Days after sowing; % decrease/increase is calculated by comparing with control (data from Sharif et al. 2005).

Another study conducted by Jamil et al. (2005) revealed that sorgaab at 6 or 12 L ha^{-1} applied in combination with lower rate of sulfosulfuron (15 g a.i. ha^{-1}) at 30 DAS reduced *Avena fatua* density (78–92%) and dry weight (72–98%) and was statistically at par with full dose of sulfosulfuron (35 g a.i. ha^{-1}). One spray of sorgaab at 12 L ha^{-1} tank mixed with sulfosulfuron (15 g a.i. ha^{-1}) at 30 DAS suppressed *Phalaris minor* density by 82–91% and dry weight by 86–87%. The corresponding suppression in weed density and dry weight with sulfosulfuron (35 g a.i. ha^{-1}) was 91–95% and 97–100% respectively. Sorgaab tank mix with reduced dose of sulfosulfuron gave as much wheat grain yield as was realized by the use of recommended dose of sulfosulfuron.

13.4 Summary and Conclusions

There exists a tremendous unrealized potential in using allelopathy in agricultural weed control. Sorghum proves to be a very effective allelopathic crop in weed management of wheat. Several water soluble potent allelochemicals are present in its leaves, stems, roots and seeds. Because of its rapid and robust growth the plant produces a large quantity of biomass very quickly. Although sorghum cultivars differ in their allelopathic properties usually the commonly used varieties in the existing cropping system release considerable amounts of allelochemicals in the rhizosphere which effectively influence weeds and the crops of successive rotation. Our studies revealed that sorghum stalks used as mulch suppress many weeds. Using sorgaab for weed inhibition and reducing herbicide dose is one of the most attractive applied aspects of sorghum allelopathy which has been practically demonstrated by research team working at our Weed Science-Allelopathy Laboratory, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan. In addition to sorghum we have been using other allelopathic crop/plants such as sunflower, *Brassica*, rice and mulberry in combination with sorgaab to determine more effective and viable weed control strategies that may reduce and in some cases replace several herbicides. While these studies produced encouraging results of weed control and crop yield the long-term effect of repeated use of sorgaab and sorghum mulch on soil fertility, microbial activity and organic matter contents have yet to be determined.

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14. Allelochemicals in Pre-cowing Soils of Continuous Soybean Cropping and Their Autointoxication

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Abstract. The problems caused by continuous cropping in soybean were discussed from both biotic stress and abiotic stress aspects; special focus was paid on origin and activity of allelochemicals under biotic stress. Interaction between phytotoxins and pernicious microorganisms in the soils of soybean under continuous cropping practice was suggested as a possible mechanism.

14.1 The Problem of Soybean Continuous Cropping

The term "continuous cropping" means a crop has been continuously cultivated for several years in the same field. In this system, after several seasons the crop may have poor growth due to serious impact of diseases and insect pests leading to low yields. Recently, the planting area of soybean (*Glycine max* [L.] Merr.) has been significantly enlarged and continuous cropping has been a serious problem in soybean production. For instance, in Heilongjiang province, the northeast of China, there was an area of 1,330,000 ha of soybean cultivation in 1970s, adding quickly up to 1,730,000 ha in 1980s, although the area dropped a bit since 1993 with 2,970,000 ha, but it was beyond 2,860,000 ha in 2000 again. Therefore the area of soybean under continuous cropping has increased steadily and accounted for 40–50%, reached 70–90%, of the whole soybeans planting area (Liu 2001; Yang et al. 1994; Yu et al. 1993).

Studies have shown that, under continuous cropping, both elongation of taproot and formation of lateral root of soybean were significantly suppressed during seed germination (Ruan et al. 2001a), and the plants had a smaller size with a poorly developed root system at seedling stage. It is concluded that the soybean root system under continuous cropping has slow development, a less quantity of fibre and a reduced root fresh weight (Xu et al. 1996). Moreover, studies have demonstrated that under continuous cropping the cells outside the root endoderm may undergo atrophy and even cell death, and lose their function of storage and transport; while under rotation system, the cells of soybean root keep integrity and arrange orderly with VA mycorrhiza inhabiting in them (Ruan 2000).

In general, continuous cropping resulted in smaller seeds, increased ratio of seeds with diseases and insect pests, leading to low commercial quality and economic yield in soybeans (Xu et al. 1996; Xu et al. 1999; Yang et al. 2001).

14.2 Mechanisms on the Problem of Continuous Cropping in Soybean

During the past two decades, the possible mechanisms on the problem of soybean monoculture have received considerable attention and have been extensively investigated. Earlier studies have shown that both biotic and abiotic stress may be involved in biodiversity decline of repeated soybean monoculture (Han and Xu 1999).

14.2.1 Abiotic Stress

Since 1990, numerous studies have been conducted on abiotic stresses caused by soybean monocropping from different aspects, such as soil physics, soil chemistry, soil biochemistry and soil fertiliy (Fu et al. 1996; Han et al. 1998a,b, 2000a; Ji and Xu 1995; Jia and Yu 1995; Jia et al. 1999; Zou et al. 1997; Yang et al. 1994; Dong and Xie 1995; Wang et al. 1999; Wang et al. 2000). The above studies still could not clarify the major mechanisms related to the problems caused by soybean monoculture. Nevertheless, it is believed that abiotic stresses have a tendency to aggravate the adverse habitat effects under continues cropping by increasing the levels of phytotoxins.

14.2.2 Biotic Stress

Biotic stress includes serious plant diseases and insect pests, unbalance of soil microbial system, and accumulation of phytotoxins in soil released from both the plant and the native microorganism. In continuous cropping system, the harmful biology especially the dominant plant diseases and insect pests such as *Heterodera glycines*, *Pythium* spp., *Fusarium* spp., *Hizoctonia* spp., and *Ophimyia shibasu* have shown clear adverse effects on soybean growth, development and yield; and the impact become worse with prolonged years of continuous cropping (Xu et al. 1998; He et al. 2003). Studies have showed that, under continuous cropping, there is an unbalance of microorganism system, i.e. the ratio between fungi and bacteria, in soybean rhizosphere, which may reduce the bioactivity of symbiotic nitrogen fixation (He et al. 2003).

Moreover, previous works revealed that autotoxic effect of phytotoxins is a dominant reason to reduce soybean yield under continues cropping (Patterson 1981; Wang and Xu 1995a,b; Yan and Yang 1998; Yan et al. 2000; Han and Xu 1998).

14.3 Allelochemicals in Soil of Replanted Soybean and Their Autotoxic Effects

In general, the great majority of autotoxic chemicals in soil are secondary metabolites, which were secreted, leached or released from living roots, leaves, stems, fruit, rhizomes, seeds, flowers, and decayed plant residues through microbial action (Putnam 1983). Both abiotic and biotic stresses have the same tendency to increase the levels of secondary metabolites (Gershenzon 1984; Einhellig 1996). Large amounts of autotoxic chemicals may accumulate in the rhizosphere and further spread unequally into the adjacent soil under continuous cropping system. Therefore, it is possible for that the following year soybean roots may come in contact with the autotoxic chemicals while they are growing in the field. Because decayed plant residues of the previous year had the most severe inhibitory effect at the early stages of decomposition (An et al. 1996), these autotoxic compounds, which come from the decomposed soybean residues are likely to inhibit both germination and early growth of soybean seedlings. In addition, plant diseases and insect pests may easily damage these weak seedlings. As a consequence, autotoxicity of these allelopathic compounds may have a detrimental effect on the yield of the successive years soybean compared to the previous years (Yan and Yang 1998). Therefore, it is necessary to identify and quantify these allelochemicals with autotoxic effect and to further understand their biological activities.

14.3.1 Allelochemicals from Soybean Root Exudation and Their Autotoxic Effects

Root exudation is an indispensable component of autotoxic chemicals and plays direct or indirect roles in affecting plant growth and development under continuous cropping (Rice 1984; Gao and Zhang 1998). Autotoxic effect of soybean root exudation on its seedling has been investigated via tissue culture under sterilized conditions, and several known allelochemicals such as vanillic acid, vanillin and p-hydroxybenzoic acid have been detected (Du and Jin 1999). By using HPLC and GC/MS, Granato et al. (1983) detected fifteen dominate peaks from root exudations of three varieties of soybean in eight different growth periods. The concentrations of those compounds were highest at root nodule formation and bloom periods and lowest at seedling and mature periods. It has been known that two isoflavonoids from soybean root secretion could induce inhibition of soybean rhizobium (d'Arcy-Lameta 1986). Studies have also shown that reduction of soybean seed germination was increased with increasing concentration of soybean root secretion (Wang et al. 2000; Wang and Xu 1995b).

Han et al. (2000b) investigated allelopathy of soybean root exudes extracted by dichloromethane and XAD-4 resin respectively, and identified compounds in these extractions through GC/MS analysis. Their results showed that allelochemicals in the dichloromethane extraction were fatty acids, phenol acids, alcohol, ester, aldehyde, and hydrocarbon; but in the XAD-4 resin they found long-chain fatty acids, benzoic acid and its derivates, straight-chain alcohol, enol, phenol, ketone, aniline, ester and some hydrocarbon. In a prescriptive rang of concentrations, one compound from them was 1,2-benzenedicarboxylic acid which may markedly inhibit seedling growth and root development in soybeans.

14.3.2 Allelochemicals from Soybean Aqueous Leachate and Their Autotoxic Effects

Leachate from plants by the action of rain, snow, fog, dew, or moist may contain majority of allelochemicals into the soil in heavy rainfall regions (Kumari and Kohli 1987). In heavy rainfall season plant leachates may accumulate to large amounts of allelochemicals in soil. The contents of vanillin, p-hydrobenzoic, vanillic, frulic and syringic acids from the aqueous leachate of soybean shoot under replant system was higher than that under rotation system. The aqueous leachate showed a similar adverse effect on the growth of soybean seedling as compared with p-hydrobenzoic under aqueous culture condition. The concentration of those compounds from replant system positively correlated with the population of fungi in soil. It has been demonstrated that all three fractions from the aqueous leachate of soybean shoot at the seedling stage exhibited a strong inhibitory effect on elongation of soybean radical (Wang et al. 1995a). Du and Jin (1999) further detected several known autotoxic chemicals such as vanillic acid, vanillin and p-hydroxybenzoic acid from aqueous leachate of soybean shoot.

Seed germination and seedling growth of soybean were depressed at 0.6 DW/ml (dry weight of soybean shoot to distilled water) by the aqueous leachate of soybean shoot. These compounds were detected by GC/MS as low weight fatty acids, phenol acids and their derivatives, benzene, alcohol, phenol, aldehyde, ketone, amine, carbonitrile, naphthalene, ester and so on, and most of them were reported as allelochemicals (Han et al. 2002).

14.3.3 Allelochemicals from Decayed Soybean Residues and Their Autotoxic Effects

It is well known that one of the main reasons for autotoxicity in some crops is due to the accumulation of phytotoxins from the decomposing residue of the previous crop (Devi et al. 1997). Depending on the residence time, persistence, concentration, and fate of the phytotoxins these organic chemicals from the decomposing residue of the previous soybean may contribute significantly to the autotoxic effects.

The root system of soybean consists of a tap-root with length 2~3 meters, attaching to many lateral branches. Most of the roots leaves and leafstalks of soybean are returned to the field in conventional agriculture system. The plant residues may weight about up to 1/3 of the whole soybean plant. In northeast of China, stubs and litter of soybean in soil were hardly decayed in the winter after soybean harvest. In the beginning of the next summer, these residues are rapidly decomposed due to increasing activity of microorganisms with rising soil temperature. After 45–60 days, the speed of decomposition reaches the maximum coinciding with more elevated soil temperature and rainfall (Chen et al. 1998). The concentration of autotoxic chemicals in soil from decayed residues would also be the highest level at this time. Because of low photosynthetic rates and an underdeveloped root system of soybean during this period, the young soybean seedlings become very sensitive to both phytotoxins and pernicious microorganism in the soil, which might have been contributed to the lowered plant growth and bean yield.

The aqueous leachates from decayed soybean residues obtained from soybean rhizosphere soil inhibited seed germination and seedling growth of soybean (Wang and Xu 1995a; Wang et al. 2000). Han and Xu (1998) showed that the inhibition started from the beginning of seed germination, and gradually abated as progress of decomposition.

Ruan et al. (2001c) detected vanillic, syringic, p-coumaric and frulic acids from the ethyl acetate fraction of decomposed soybean root residues. Huang (2001) found three isoflavonoids such as daidzein, genistein and coumestrol in soil solution from rotation and continuous cropping system, and proved that these isoflavonoids were derived from decomposition of soybean residues. Further investigations with bioassay showed that daidzein at a concentration of 4 μg/ml coumestrol at 0.64 μg/ml in nutrition solution could inhibit the growth of soybean seedling.

Han et al. (2000c) found that all of the acidic and alkaline fractions extracted from soybean root stubs, under decomposition for two weeks, four weeks and eight weeks, respectively, with rhizosphere soil taken as inoculants, significantly inhibited seed germination and radical growth of soybean. The autotoxic effect of the compounds in the acidic fraction was stronger than those in the alkaline fractions. With Trichode rmakoningii as inoculants to decompose root stubs of soybean for two weeks and four weeks, the components extracted by cyclohexane, ethyl acetate and XAD-4 resin showed immediate inhibitory effect in the initial stage of soybean seed germination (21 h), and the inhibition become weak thereafter. These authors concluded that the chemicals from soybean decayed root stubs had a significant autotoxic effect on seed germination, radical elongation and development of soybean, and all these effects may eventually cause reduction of seed yield.

Through seed germination and early seedling growth bioassay, we analyzed inhibitors in every faction separated from the aqueous extract of decayed soybean residues, and detected twelve compounds from the most toxic fractions (Fig. 1) (Yan 2003). In a culture experiment of soybean seedlings, we also found that 8-octadecenoic acid methyl ester alone did not suppress the early growth of soybean, whereas, with increased concentration of phenol 2,4-bis (1,1-dimethylethyl) alone, stem elongation was inhibited and the damage on cell membrane of root was significant. However, there were significant synergistic effects in the mixture of Phenol 2, 4-bis (1,1-dimethylethyl) and 8-octadecenoic acid methyl ester at V Phenol/V ester=1:1 and 3:7 with 1×10^{-3} mol·L^{-1}. We concluded that autotoxic activities of soybean may originate from mixtures of autotoxic compounds, and that the concentration of each compounds in a mixture might be markedly less than that of individual compounds required to reduce an autotoxic effect (Yan 2003).

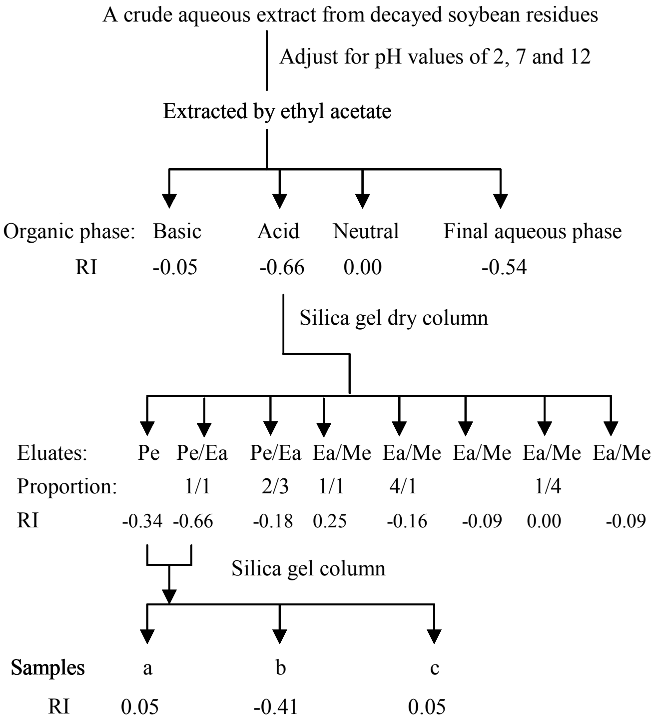

Fig. 1. Isolation procedure for allelopathic compounds from the decomposition products of decayed soybean residues

Note: RI means response index; Pe, Ea, and Me mean petroleum ether, ethyl acetate and methanol, respectively.

14.3.4 Allelochemicals in Soybean Rhizosphere Soil

By sterilizing soybean replanted soil with CH_3Br, Ruan et al. (2001b) found that soil inhibitory chemicals were present in the soybean residue and their decomposition products, which confirmed that the autotoxic effect was existed in the soybean replanting system. The result of the bioassay further proved that there were autotoxic allelochemicals present in soybean soil (Yan et al. 2000). Zhang et al. (2000) found that, under replant system, the content of p-hydrobenzoic and vanillic acids, which were extracted from soybean soil with 1 mol/L NaOH, was significantly higher than that under rotation system. After a week when these six compounds had been added into the soil, the residual ratio of vanillin, p-hydrobenzoic, vanillic, hydrobenzoic, frulic and syringic acids were 4.1, 10.4, 15.3, 2.3, 5.0 and 7.5%, respectively.

The allelochemicals from the sick soil of soybean under continuous cropping played a key role to inhibit emergence and development of soybean. This maybe owing to the chemical transformation effect of microorganisms in soil on some organic compounds from soybean root exudes, decayed stubs and shoot leachate (Han et al. 2000d).

14.3.5 Interaction Between Soil Bacteria and Allelochemicals in the Soil

Soil microorganisms play an important role in allelopathy by influencing the release of chemical compounds bound or adsorbed to soil particles and degrading allelopathic chemicals producing either less or more toxic by-products. These allelochemicals modified by microorganisms can impact on the plant uptake of nutrients from soil, which may further affect the fate of allelopathic chemicals. Correspondingly, some allelopathic chemicals have also the potential to stimulate soil microorganisms and pathogenic fungi. The pathogenesity in combination with allelopathic effects may have more adverse effects on the plants in comparison to either effects alone.

Large numbers of microorganisms in soil not only decompose plant residues and produce allelochemicals but also produce plant metabolites. Wyllie and Morehouse (1978) found that the inhibitor from Fusarium oxporum depressed soybean growth and metabolites of *Rhizoctonia solani*, which itself did not infect soybean, but strongly inhibited the seedling growth. Hu and Wang (1996) observed *Penicillium purpurogenum Stoll* 891 from dominant fungi in soils of soybean under continuous cropping system, and its crude crystals can injure soybean root system when the concentration reached 5 g/ml in water culture. Stimulating effect on several dominant pathogenic fungi of root rot from soybean root exudates under continuous system was stronger than that under rotation system. However, different genotypes of soybean showed different allelopathy on these pathogenic root rot fungi (Jia et al. 1997; Ju and Han 2002).

14.4 Conclusions

We suggest that autotoxic chemicals that mainly derived from decaying soybean residues exhibit initially the most severe inhibition in field, poor seedling emergence and growth. As decomposition proceeds, the inhibitory effects of phytotoxins decline. But subsequently soybean diseases and pests maybe sharply aggravated, which would likely be attributed to the stimulatory effect of phytotoxins on harmful microorganisms with the rise of soil temperature and moisture. The end results of autotoxicity associated with continuous cropping are manifested in soybean yield decline (Fig. 2). We predicate that the depressed root at the early stage is a primary factor, but that does not account for total inhibitory effects. It is most likely regulated by the interactions between phytotoxins and pernicious microorganism.

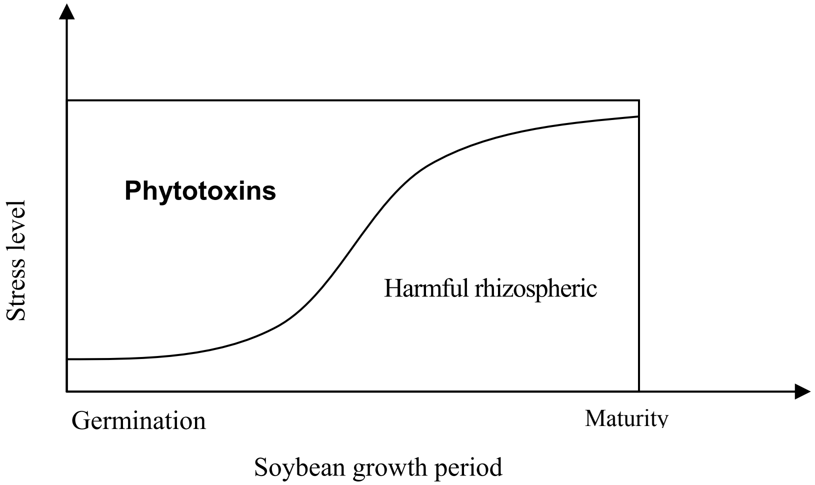

Fig. 2. Change tendency of the dominant stress factors in the problem of soybean continuous cropping

Although hundreds of allelochemicals detected in pre-cowing soils of continuous cropping soybean, only a few of them may possess significant biological activity. Therefore, it is important to identify and quantify the toxic compounds from them, and further analyze their autotoxic effects in the problem of soybean monocropping system. It is clear that a better understanding of autotoxic effect would help resolve the problem aiding the progress toward a more sustainable agriculture.

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15. Autotoxicity in Agriculture and Forestry

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Abstract. Plant allelochemicals might interfere with growth of conspecific individuals (known as autotoxicity) when high concentrations are accumulated in soil. Autotoxicity is ubiquitous in both natural and manipulated ecosystems and may have important ecological implications. This chapter reviewed the role and management of autotoxicity in agriculture and forestry. It also discussed the mode of action of autotoxicity and its controlling factors.

15.1 Introduction

Plants produce a wide variety of allelochemicals to defend themselves from herbivores and microbial pathogens (Verpoorte et al. 1999). Many of these chemicals are also used to interfere with other plants in their vicinity (Rice 1984). The phenomenon by which one plant directly or indirectly cause detrimental (or occasionally beneficial) effects on other plants through the release of allelochemicals into the environment is called allelopathy (Rice 1984). Allelopathic effects are mostly negative (Inderjit and Callaway 2003) but in rare cases the effects can be beneficial (Callaway 1995). Allelochemicals may be released by plant leaching, root exudation, volatilization and residue decomposition (Rice 1984). These allelochemicals not only cause growth interference among different species (interspecies) but they can also cause growth interference of the same species (conspecific) when high concentrations are accumulated in soil. Putnam (1985) defined autotoxicity as a process where a plant or its decomposing residues release toxic chemicals into the environment to inhibit germination and growth of the same plant species.

Continuous monocropping and intensive culture systems often result in poor growth and yield decline of crops known as soil sickness. In agroecosystems, soil sickness has been a commonly known phenomenon for many centuries. Clover soil sickness was known in Europe in the 17th Century (Katznëlson 1972). In 1832, De Candolle, a Swiss botanist, suggested that the soil sickness problem in agriculture might be due to autotoxicity of exudates of crop plants (Jackson and Willemsen 1976). Autotoxicity has been related to many problems in agriculture and forestry systems, e.g., soil sickness, continuous cropping obstacles, orchard replanting problems, and forest regeneration etc. Schreiner and Reed claimed in 1907 that the roots of wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.) and other crop plants exude chemicals inhibitory to their own seedlings (Jennings and Nelson 1998). Many agricultural crops such as rice (*Oryza sativa* L.) (Chon et al. 2000; Chou and Lin 1976; Chou and Chiou 1979), wheat (Einhellig 2004; Kim and Shin 1998; Lin et al. 2005a), alfalfa (*Medicago sativa* L.) (Chen et al. 2002; Hirano 1940), barley (*Hordeum vulgare* L.) (Ben-Hammouda et al. 2002), ryegrass (*Lolium perenne* L.) (Fageria and Baligar 2003), corn (*Zea mays* L.) (Lin et al. 2005a,b), mungbean (*Vigna radiata*) (Kim and Kil 2001), *Asparagus officinalis* (Kato-Noguchi 2004; Loffredo and Senesi 2006), tomato (*Lycopersicon esculentum*) (Zhou et al. 1997), lettuce (*Lactuca sativa* L.) (Khafagi 1998), cucumber (*Cucumis sativus*) (Yu and Matsui 1994; Luo et al. 1995; Martin and Leonard 1967), sunflower (Maruthi and Sankaran 2001), marigold (Karin and Kaui 2000) and goldenrod (Ikutaro et al. 1998) have been reported to exhibit strong autotoxicity. Replant problem is also very prevalent in orchards of apple, peach, citrus, grape, cherry, strawberry, and rose. Tea tree (*Camellia sinensis*) (Cao and Luo 1996), coffee (*Coffea arabica*) (Anaya et al. 2002; Friedman and Waller 1983), Chinese fir (*Cunninghamia lanceolata*) (Zhang 1993, 1997), Horsetail beefwood (*Casuarina equisetifolia*) (Deng et al. 1996; Batish et al. 2001b), *Pinus massoniana* (Huang et al. 1995), *Picea schrenkiana* (Luo et al. 2006) were found with autotoxicity. It is often difficult to grow new trees after cutting the old ones. Soils in orchards can be very toxic compared with the non-orchard soils. Besides toxicity to microbes, pathogens, and nematodes, autotoxicity is believed to play a key role in causing replant syndrome (Brinker and Creasy 1988; Singh et al. 1999). Tea yield decreases rapidly in late growing stages of tea tree (Wang 1990). The internodes of apple trees grow slowly in the old orchards. A number of phenolic acids and flavonoids in the leaves, bark, and fruit of apples are responsible for the replant problems (Williams 1960). The replant problem observed in grape nursery could not be reversed by fumigation, fertilization, and cover crops and pathogens were excluded as a cause (Brinker and Creasy 1988). The replanting problem in Chinese fir is very common (Huang et al. 2000; Huang et al. 2002). Trees release allelochemicals through the degradation of litter, root exudates, and bark. For example *Picea abies* release many phenolic acids and tannins which inhibit the germination, growth, and establishment of young seedlings (Gallet 1994).

Autotoxicity is a common phenomenon in both natural and manipulated ecosystems and may have important ecological implications, minimizing intraspecific competition, extending seed dispersal, regulating plant population over space and time, and maintaining seed dormancy over a long period of time in stressful environments (Liu et al. 2007; Singh et al. 1999). Autotoxicity can be a driving force in succession of some plant community (Jackson and Willemsen 1976; Quinn 1974).

Autotoxicity could be important in pure stands of long term crops and some continuous cropping systems. Continuous cropping is not recommended because of potential problems due to soil water depletion, plant diseases, and autotoxicity (Batish et al. 2001a; Huang et al. 2000; Kimber 1967). This chapter briefly reviews the role, autotoxins involved, ecological implications and management of autotoxicity in agriculture and forestry.

15.2 Autotoxicity in Agriculture and Forestry

15.2.1 Leguminous Plants

15.2.1.1 Alfalfa (Medicago sativa)

Alfalfa is one of the most important and oldest forage crops in the United States. It has a very high yield potential and highest feeding values. However, autotoxicity in established alfalfa fields can cause low germination, poor growth and extensive death of new alfalfa through overseeding. In 1909, Wing reported a reduction of alfalfa yield in rotation and difficulty of re-establishment occurred in fields (Wing 1909). Jensen et al. (1981) first described autotoxicity of alfalfa during and after alfalfa establishment. Recent research has shown that the chemical exudates from alfalfa caused the “soil sickness” problem and contributed to the long-term reductions in alfalfa plants (Klein and Miller 1980; Miller 1983). Various toxic compounds potentially involved in autotoxicity were localized in the seed coat, fresh alfalfa leaves, stems, crowns, dry hay, old roots, and soil residues (Miller 1983). Hegde and Miller (1992) showed that several phenolic compounds exerted phytotoxicity on the root and shoot growth of alfalfa. Water-soluble toxic substances in alfalfa inhibited the seed germination and seedling growth of alfalfa (Chung and Miller 1995a). Chon and Kim (2002) identified nine phenolic compounds in alfalfa plants. Caffeic, ferulic and *p*-coumaric acids were detected in all plant parts, *o*-coumaric and salicylic acids were present in the leaves and seeds, and *trans*-cinnamic acid was found in the leaves and stems.

15.2.1.2 Soybean (Glycine max)

Continuous cropping of soybean has resulted in the serious problems of yield decline after several seasons, which was, apart from diseases and insect pests, related to the allelopathic autotoxic effect (Petterson 1981; Han et al. 2000a,b). Chen et al. (1998) reported that in northeast China the stubs and litters of soybean in soil hardly decayed in the winter but rapidly decomposed in the beginning of the next summer, as the activity of microorganisms increased with soil temperature rising. Some compounds were identified from the decomposed soybean stubs, the root exudates and plant aqueous extracts of soybean. They included organic acids, alcohol, ester, acetone, aldehyde, phenol, pheyl, hydrocarbon, vanillic acid, vanillin, *p*-hydroxybenzoic acid, etc., and inhibited the soybean seedling growth, and damaged the structure of biomembranes (Du and Jin 1999; Han et al. 2000b). The soybean growth is strongly inhibited by *Penicillium purpurogenum*, a dominant fungus in soil of soybean continuous cropping (Hu and Wang 1996). Soil sickness of continuous cropping soybean could be ameliorated by soil sterilization (Yu et al. 1988).

15.2.2 Solanaceae

15.2.2.1 Tomato (Lycopersicon esculentum)

Tomato is an important vegetable crop in the world. Six decades ago, Hirano (1940) found that soil problems caused the decrease of tomato production. Tomato has allelopathic potential (Kim 1987; Kim and Kil 1989; Kim and Kil 2001), recently study also shown it has strong autotoxicity. Eight years of continuous cropping in greenhouse resulted in a significant reduction in root vitality and fruit quality of tomato plants (Wu et al. 1997). Zhou et al. (1997) found the leaf leachates of tomato plants significantly inhibited the root growth of tomato at the concentrations of 0.05 g FW (fresh weight) mL^{-1} or higher and the shoot growth at a concentration of 0.1 g FW mL^{-1}. Root exudates of tomato in hydroponic culture inhibited tomato shoot height and fresh weight by 17 and 37%, respectively, in comparison with control. Root exudates of tomato plants in hydroponics under controlled climatic conditions showed strong autotoxicity and allelopathic potential against lettuce, and the factors such as soil humic and fulvic acids were shown to intensify toxicity of tomato root exudates (Loffredo and Senesi 2006).

15.2.2.2 Eggplant (Solanum melongena)

The multiple cropping index of eggplant is very high. It has been documented that eggplants do not grow well if cultivated consecutively for several years on the same land and its residues inhibited the growth of eggplant (Yu et al. 2000). Wang and Wang (2005) showed that the aqueous extracts from eggplant residues have strong autotoxic effects on eggplant. The leaf leachates of eggplant significantly inhibited the plant radicle growth and almost stopped the radicle growth at a concentration of 0.075 g/mL. Although the growth of embryo was stimulated at a lower concentration, the inhibition occurred at the concentration of 0.075 g/mL. Methanol isolatable components in the aqueous extracts also inhibited the growth of embryo, radicle and seedling (Wang and Wang 2005).

15.2.3 Cucurbit Crops

Cucurbit crops are important vegetables worldwide. However, they are vulnerable to soil chemistry (Yu et al. 2000), and autotoxicity is widespread in many cucurbit crops, e.g., cucumber (*C. sativus*), muskmelon (*Cucumis melo*), and watermelon (*Cucumis lanatus*) (Yu and Matsui 1994, 1997; Yu et al. 2000; Yu 2001).

15.2.3.1 Cucumber (Cucumis sativus)

Allelopathic effects of cucumber plants on some weeds were reported by Putnum and Duke (1974) and Lockerman and Putnam (1979, 1981). Autotoxicity in cucumber plants has been reported by Politycka et al. (1984). Research has shown that cucumber root exudates significantly inhibited its own seed germination and seedling growth (Asao et al. 1998a), and also decreased fruit yield (Asao et al. 1998b). The addition of activated charcoal in nutrient solution in hydroponic culture significantly improved the growth and yield of cucumber (Asao et al. 1998b; Pramanik et al. 2000). Decomposition of cucumber straw inhibited cucumber seed germination but stimulated seedling growth (Feng et al. 2005). The autointoxication mainly occurred in the late period of cucumber growth (Yu and Matsui 1994). The autotoxic compounds contained in cucumber root exudates were mainly phenolics. Benzoic, *p*-hydroxybenzoic, 2,5-dihydroxy-benzoic, 3-phenylpropionic, cinnamic, *p*-hydroxy cinnamic, myristic, palmitic, and stearic acids, as well as *p*-thiocyanatophenol were identified in root exudates of cucumber plant (Yu and Matsui 1994). Benzoic, cinnamic,and *p*-hydroxybenzoic acids directly hindered the nutrient and water utilization of cucumber (Wu et al. 2002). The high concentrations of cinnamic acid damaged the structure and the function of plasmalemma of cucumber seedling, but induced cucumber seedlings to adapt the autotoxicity (Wu and Ma 2006). Although cucumber grafting on squash (*Cucurbita moschata*) may remove the autotoxic effect, root exudates of squash grafted had strong allelopathic effects to cucumber (Yu 1999; Yu et al. 2000).

15.2.3.2 Watermelon (Cucumis lanatus)

Watermelon is vulnerable to soil sickness (Yu 1999). In continuous watermelon cropping systems, autotoxicity is the main reason for yield decrease rather than nutrient depletion and competitive effects. Aqueous and methanol extracts decreased the radicle elongation of watermelon but the effects on lettuce were not significant (Hao et al. 2006). The extraction of root, stem and leaf of watermelon has inhibitory effects on seed germination and enzyme activities of watermelon seedlings (Yang et al. 2005). Root exudates restrained seed germination and seedling growth after monoculture (Yang et al. 2005). The phenolic acids including cinnamic acid, *p*-coumaric acid, ferulic acid and vanillic acid were identified from the root exudates and root residues of watermelon (Yang et al. 2004a). α-Hydroxybenzoic acid was isolated from the root residue (Yang et al. 2005). Watermelon autototoxic effect was related to different growth stages. Seed germination and seedling stage were particularly sensitive to autotoxic chemicals (Yang et al. 2005).

15.2.4 Gramineous Crops

15.2.4.1 Rice (Oryza sativa)

Rice is the staple grain in many Asian countries. Its allelopathic potentials have recently attained worldwide attention since it provides a sustainable approach to control weeds in paddy field (Dilday et al. 1998). However, autotoxicty can not be ignored in the second rice growing season in many Asian countries. In Taiwan yield reduction in the second crop rice due to autotoxicity of rice residues may reach 25% in areas of poor water drainage (Chou 1990). Usually rice is cultivated twice a year in tropical and sub tropical areas and rice straws are used for nutrient recycle in the same field by many farmers. These returned rice straws plus un-harvested rice stubble are decomposed in the field in anaerobic condition and release large amounts of phenolic acids such as *p*-hydroxybenzoic, *p*-coumaric, syringic vanillic, ferulic, and *o*-hydroxyphenylacetic acids and other autotoxins, which result in significant growth stunt and yield decrease in successive rice plants (Chou and Lin 1976; Chou 1986). The concentration of *o*-hydroxyphenylacetic acid reached 10^{-2} M in the first week of the decomposition period, and this compound display inhibitory effects on the radicle growth of rice at only 25 ppm (Chou and Lin 1976). The accumulation of autotoxins during decomposition of rice residues in soil reached highest levels six weeks after incubation, gradually declined thereafter and disappeared after twelve weeks (Chou et al. 1981). The water-soluble phenolics degraded from rice straw also showed allelopathic effects on other species (Inderjit et al. 2004). Appropriate management of autotoxicity of rice straw may minimize the detrimental effects of first rice crop on the second cropping.

15.2.4.2 Sugarcane (Saccharum officinarum)

Sugarcane is grown in the tropical and subtropical regions. In many regions, continuous cropping of sugarcane is very common, which often leads to the crop yield reduction (Chou 1995). Autotoxicity has been identified as one component of such reductions (Sampietro 2006). Extract of sugarcane stubble residues increased sugarcane bud germination at a lower concentration, but germination, radical length and seedling dry weights were reduced at all concentrations (Sampietro 2006). Autotoxicity of sugarcane delayed its early leaf development (Johnson et al. 2006). The decomposing sugarcane residues in the fields release various phenolic acids and fatty acids into soil (Chou 1995).

15.2.5 Autotoxicity in Orchards

15.2.5.1 Tea Tree (Camellia sinensis)

Tea tree is originated from China and it is one of the most important crops in the hilly and mountainous area of southern China. However, it has been found that tea productivity decreases rapidly over time after the initial establishment of tea gardens and is affecting the economy of tea farmers (Wang 1990). Regeneration in old tea plantation was also difficult (Cao and Luo 1996). Recent studies indicated that autotoxicity may play an important role in population deterioration and yield reduction in the later growth stages. Tea plants contain many important polyphenols with antioxidant properties (Guo et al. 1996). The high content of caffeine in tea plants may be involved in allelopathy (Suzuki and Waller 1987). Polyphenols and caffeine abundant in tea plants significantly inhibited seed germination and seedling growth of tea tree (Cao and Luo 1996). Root exudates of tea tree and aqueous extracts from various plant parts significantly inhibited seed germination, and root and shoot growth of tea plants (Cao and Luo 1996). Tea also contains many biologically active catechins, which made up to 30% of the dry weight of its leaves (Ahmad and Mukhtar 1999). These catechins include epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate (EGCG), with EGCG being the principal constituent. The later compound influences the growth of both inter- and intraspecific plants and may act as both an allelochemical and an autotoxin (Perry et al. 2005).

15.2.6 Autotoxicity in Planted Forests

15.2.6.1 Chinese Fir (Cunninghamia lanceolata)

Chinese fir is one of the most important fast-growing conifers established widely in southern China. However, serious problems with productivity decline have been widely experienced after replanting of Chinese-fir on the same site in successive rotations. The failure of growth of Chinese fir seedlings in replant woodland was caused by several factors including depletion of nutrients, deterioration of the structure of replant woodland soil, some biotic factors and particularly allelopathy (Chen et al. 2002; Huang et al. 2002; Zhang 1993, 1997). Zhang (1993) found that extracts of soils collected from the second and the third rotation of Chinese fir replant woodlands significantly reduced the growth of Chinese fir seedlings. Using a hydrophonic culture system, Zhang (1993) demonstrated that the extracts of decomposing root residues collected from both the first and second replant woodland showed significant effects on the growth of height, diameter at ground level, and the accumulation of aboveground and belowground dry weight of Chinese fir. The combination of the decomposing root residues and the pathogenic fungi caused the greatest growth reduction of seedlings (Zhang 1993). Subsequent work demonstrated that soil allelopathy, particularly autotoxicity, rather than pathogenic fungi was the key factor regulating the productivity and nutrient cycling in repeatedly planted woodlands of Chinese fir (Huang et al. 2000, 2002; Zhang and Yu 2001).

In an attempt to search for responsible agents for poor establishment of Chinese fir, Huang et al. (2000) examined the changes in total phenolic content in decomposing stump-roots of Chinese fir at the different ages in replanted woodland. They found that the phenolic content was the highest in roots, followed by stump heartwood and sapwood, while the total phenolic content in stump-roots decreased with increasing stump-root age. Subsequently eight phenolic compounds including gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, *p*-coumaric, *m*-coumaric, syringic, and ferulic acids were identified in the Chinese fir stump-roots (Huang et al. 2000). Bioassay results showed that phenolics released from the Chinese fir stump-roots reduced the growth of its own seedlings and the total weight of seedlings was negatively correlated with total phenolic content in the stump-roots that had been added to the soil. In pot experiments, fir root, fresh leaves and litter placed on the soil surface significantly reduced its own seedling growth (Huang et al. 2002). The Chinese fir seed germination was significantly inhibited by 10% (w/v) aqueous extracts of fresh leaves and litter or as low as 1% aqueous extract of fresh roots of Chinese fir, while at the low concentration of 0.1% aqueous extract of bark the seed germination was significantly stimulated (Huang et al. 2002).

15.2.6.2 Horsetail Beefwood (Casuarina equisetifolia)

Horsetail beefwood is widely cultivated in coastal areas of China and India for landscaping, windbreaks and sand-shifting control as well as for pulp, lumber and tannin (Doran and Hall 1983). It is an important pioneer species in coastal areas because of its salt and drought tolerance, fast growth and suitability for typhoon, sea tide and sand-shifting control. Field investigations in Nanhai Town, Dianbai County of Guangdong Province, showed that the soil texture and ecological environment had improved after planting *C. equisetifolia* (Deng et al. 1996). However, tree degradation and regeneration failure have become a serious problem since the first generation of *C. equisetifolia* has reached the turnover time (Chen and Lin 1997; He 1998). Vegetation cover under the tree is significantly lower compared to adjoining grassland area. This may be caused by potent allelopathy of this species (Batish et al. 2001b). Branchlet leachates of the plant strongly interfered with the root growth of its own seedlings (Chou 1986). Lin et al. (2005a,b) also reported that the water extract of *C. equisetifolia* roots inhibited the growth and nutrient absorption of its own seedlings. The autotoxins of *C. equisetifolia* were isolated and identified as kaempferol-3-α-rhamnoside, quercetin-3-α-arabinoside and luteolin-3',4'-dimethoxy-7-β-rhamnoside (Deng et al. 1996). Phenolic compounds released from green needles and litter of the tree inhibited under storey vegetation (Batish et al. 2001b).

15.3 Autotoxicity Mechanisms

15.3.1 Dose-Response of Autotoxic Compounds

Autotoxicity is one kind of special allelopathy and it is dose-dependent. According to Molisch (1937), allelopathy includes both harmful and beneficial biochemical effects of one plant on neighboring plants. Many studies have shown that allelochemicals can stimulate plant growth at low concentrations but inhibit plant growth at high concentrations (Rice 1984; Zeng et al. 2001). Watermelon seed germination was inhibited, but elongation of radicle and hypocotyls were stimulated by a low concentration of autotoxic compounds (Geng et al. 2005). Yang et al. (2005) showed that root, stem and leaf extracts of watermelon have some inhibitory effects on enzyme activities of seedlings. The inhibition became stronger as extract concentration increased. Aqueous extracts from eggplant residues stimulated the embryo growth at 0.015 and 0.0250 g/mL, but showed inhibitory when the concentration became higher than 0.075 g/mL (Wang and Wang 2005).

Li et al. (2005) also showed the effect of seedling growth of pumpkin (*C. moschata*) was inhibited at higher concentrations of root exudates but stimulated at a lower concentration.

Wedelia chinensis is a plant with strong allelopathic potential. Other plant species were very sensitive to the aqueous extract of *W. chinensis* plants, which often stimulated stem germination and seedling growth of its own at low concentrations. *W. chinensis* plants were very resistant to their own autotoxins and its seedling growth was only inhibited at very high concentrations (Zeng et al. 1994). Field investigation found that *W. chinensis* tended to form a pure community with few other weeds, but after approximately ten years of growth, more weeds started to grow in the community. This may have been caused by the high accumulation of autotoxins in the soil resulting in its population deterioration.

15.3.2 Modes of Action

Recent research has shown that several modes of action for allelochemicals are involved in the inhibition and modification of plant growth and development. Phenolic acids inhibit plant growth through multiple physiological effects that confer a generalized cytotoxicity (Einhellig 2004). Their initial actions are on cell membranes, resulting in non-specific permeability changes that alter ion fluxes and hydraulic conductivity of root. A cascade of physiological effects that include alterations in ion balance, plant–water relationship, stomatal function and rate of photosynthesis and respiration follow membrane perturbations (Einhellig et al. 1985; Gerald et al. 1992). These phenolics also interact with several phytohormones and enzymes, causing deviation from typical patterns for biosynthesis and flow of carbon into metabolites (Einhellig 2004). Yang et al. (2004b) reported that the three phenolics commonly found in the decomposing rice straw, ferulic, *p*-coumaric, *o*-hydroxyphenylacetic acids, may affect the biosynthetic and degradative pathways of chlorophyll. These autotoxins influence ion uptake, membrane permeability, photosynthesis and phytohormone balance in these plants. Chou and Chiou (1979) reported that the incorporation of rice straw decreased both the availables nitrogen and the soil cations, Zn^{2+}, Cu^{2+}, Ca^{2+}, Mn^{2+} and Na^{+}. The quantity of Zn^{2+}, Cu^{2+}, Mg^{2+} and Na^{+} was significantly lower in the second crop than that in the first. Autotoxins in eggplant also suppressed the ion uptake of NO_3^{-}, PO_4^{3-}, K^{+}, Mg^{2+} and Ca^{2+} (Wang and Wang 2005).

Autotoxins in cucumber reduce photosynthesis and activities of antioxidant enzymes in the crop (Yu et al. 2003). Cinnamic acid, a principal autotoxin in the root exudates of cucumber significantly decreased net photosynthetic rate, stomatal conductance and the quantum yield of Photosystem II photochemistry. Furthermore, it promoted incidence of *Fusarium* wilt in cucumber (Ye et al. 2004).

Caffeine in tea and coffee reduced the elongation of hypocotyls and inhibited the growth of rootlets (Friedman and Waller 1983). One potent mode of action of caffeine is arresting cell division by inhibiting cell plate formation. Caffeine may act by incorporation into the nucleic acid chains and thus interfere with normal cell division (Friedman and Waller 1983). It also causes a series of irregularities in root cells of corn (Anaya et al. 2002).

15.4 Factors Controlling Autotoxicity

Many factors such as crop variety, planting density, plant age, temperature, light, water, soil structure, and cultivation treatments affect autotoxicity (Chon et al. 2000; Chou and Chiou 1979; Chou et al. 1981; Chung and Miller 1995b; Deng et al. 1996; Jennings and Nelson 1998; Pramanik et al. 2000; Seguin et al. 2002). Soil microorganisms often mediate the fate of autotoxins (Blum et al. 2000; Han et al. 2001b; Hu and Wang 1996). In the paddy field, the phenolic acid can also accumulate for long time due to poor aeration (Chou 1995). The temperature of soil can influence the phenolic acid too (Pramanik et al. 2000). Location where the experiment is conducted and year in which the alfalfa is sown also influence conclusions regarding autotoxicity (Seguin et al. 2002). For example, alfalfa stands were reduced about 20% both years at Location 1; unaffected in Year 1, but reduced 10% in Year 2 at Location 2; and reduced 10% in Year 1, while increasing 15% in Year 2 at Location 3. Different growth stages of alfafa showed different autotoxicity. Alfafa at the reproductive stage contains more autotoxin than does the plant at the vegetative growth stage (Hegde and Miller 1992). Alfalfa residue after soil amendment showed strong inhibitory effects on barnyardgrass and monochoria growth (80–100% weed control) for up to 10 days (Xuan et al. 2005). After 20–25 days, the magnitude of inhibition was drastically reduced but was still noticeable. The concentrations of phenolic acids reached maximum after 10–15 days of residue incorporation into the soil, and these phenolics were detected in the soil even after 50 days (Xuan et al. 2005).

15.5 Ecological Implications

Autotoxicity has important ecological implications including dispersing plant seeds and distribution, avoiding intraspecific competition and regulating their populations over space and time (Singh et al. 1999). Maintaining high levels of autotoxins in seeds not only prevents seed germination under stressed environments, but also help resist pathogen infection and animal feeding. Autotoxicity of living plants keeps distance among individuals and avoids overcrowding and intraspecific competition for light, water and nutrients.

Autotoxicity plays an important role in community succession. Vegetation changes in a community are at least partially mediated by autotoxicity (Jackson and Willemsen 1976; Quinn 1974). Several plants in desert produce autotoxicity under drought stress in order to keep individuals alive to utilize the limited water resource.

The existence of autotoxicity in natural ecosystems may be a strategy for plants to decrease the number of intraspecific plants under poor conditions.

The mechanisms of *C. equisetifolia* deterioration are not fully known but it has generally been considered that exhaustion of mineral components and plant diseases are factors responsible for the population deterioration. A growing number of studies suggest that autotoxicity is another important potential mechanism controlling the deterioration (Deng et al. 1996; Lin et al. 2005a,b). Low biodiversity underneath the canopy of *C. equisetifolia* as well as land degradation in plantations of this species may be caused by accumulation of phenolics and some flavonoids in the soil from litter, root exudates and water leachates (Batish et al. 2001b; Deng et al. 1996), that may even cause regeneration failure. Therefore autotoxicity may be an important driving force for population replacement and community succession.

15.6 Management

Although autotoxicity is an evolutionary mechanism constructing community, it causes yield decline in agriculture, regeneration failure and replant problems in forestry. Better understanding of crop autotoxicity will provide useful information for management of soil sickness and chemistry, as well as minimize the autotoxic effects on successive crops. Autotoxicity may be overcome by manipulating population density control, appropriate crop rotation, residue management, and resistant crop varieties (Martin and Leonard 1967; Miller 1996; Singh et al. 1999).

Monoculture in agriculture is not recommended because of potential problems including soil water depletion, plant diseases and autotoxin accumulation (Batish et al. 2001a; Kimber 1967; Miller 1996). Crop rotation has been recommended as an effective soil management practice to overcome autotoxicity. China has a long history of using crop rotation, relay cropping and intercropping in agriculture practice to avoid crop autotoxicity. Strategies for overcoming autotoxicity may vary with crops and soil types. Practical and effective approaches should be investigated and adopted to avoid autotoxicity problems in different continuous crop production systems. Rice autotoxicity can be largely ameliorated by an effective management, including proper crop rotation to reduce the accumulation of autotoxins, selection of crops and varieties tolerant to autotoxins, water draining, water flooding, nitrogen dressing, detoxification of phytotoxins by polymerisation of humic substances, addition of activated charcoal, and use of lime to adjust soil pH (Chou 1986; Fageria and Baligar 2003; Inderjit et al. 2004). In addition, reducing the amount of rice straw retained in the field is a practical option to manage rice autotoxicity. The phytotoxicity and phenolic contents increased proportionally to the amount of rice straw incorporated (Chou and Lin 1976; Chou and Chiou 1979; Inderjit et al. 2004). Rice yields can also be improved by growing rice cultivars tolerant to autotoxins. Although differential responses of rice varieties to rice autotoxicity have not been demonstrated, research from other crops has shown that substantial variations in varietal sensitivities to allelochemicals exist in crop germplasm (Chung and Miller 1995b; Herrin et al.

1986). Rotation of tolerant rice varieties as the following crop is an important strategy for avoiding the autotoxic effects.

Organic manure is effective for reducing barrier of continuous cucumber cropping caused by autotoxicity. Selecting a resistant genotype, proper rotation, proper soil and plant residues management, grafting, adsorbent use and microbial degrading are suggested for overcoming autotoxicity in cucurbit crops (Yu 2001). Cao and Luo (1994) suggested using organic manure and lime properly could remove the autotoxic effects of tea. Lu et al. (2002) compared the different effects between using the organic manure and fertilizer. The results show that applying organic manure can not only relieve the inhibition of cinnamic acid on the continuous cropping cucumber but also promote its growth by improving root dehydrogenase, ATPase and microorganism activities and nutrient uptake.

The potent methods for solving soil problems in vegetable crops include introduction of antagonistic bacteria, inoculation of beneficial microbes, addition of allelopathic substances and the combination of disease resistant and disease susceptible soils (Zhen et al. 2004). Grafting could be used to overcome replant problems of vegetables and fruit trees (Zhang et al. 2005). The root exudates from grafted eggplants promoted the seed germination and seedling growth of eggplants, e.g., the germination rate and plant height increased 29.1 and 37.1% in comparison with the control. Grafting can alleviate the autotoxic effects of eggplant (Zhang et al. 2005). Fertilization management, periodic change of hydroponic nutrient solutions, and addition of activated charcoal and other absorbants to remove autotoxins from the hydroponic culture are effective. In addition, orchard cleaning, deep cultivation and application of calcium carbonate are good choices to overcome the degradation of tea plantation (Anaya et al. 2002).

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16. Black Walnut Allelopathy: Implications for Intercropping

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Abstract. Black Walnut (*Juglans nigra* L.) produces the chemical juglone that has an allelopathic effect on some companion species in intercropped systems. Nevertheless, black walnut continues to be a popular planted species in alley cropping, silvopastoral, and mixed species systems. Recent advances in management techniques, including the use of polyethylene root barriers, trenching or disking, and planting and managing the companion species during the early establishment of black walnut, have proven to be effective in alleviating the allelopathic effects of juglone. While allelopathic interference is a reality in black walnut intercropped systems, management intervention may help sustain the production potential so that they become an economically viable option for landowners.

16.1 Introduction

Walnut species (*Juglans* spp.) have intrigued researchers and the general public alike as an allelopathic species for at least the last 2 millennia. Pliny the Elder in about 1 AD wrote in his *Naturalis Historia* that ‘the shade of the walnut even caused headaches in man and injury to anything planted in the vicinity’. Similar observations have been reported by several authors during the Medieval period and in the modern history. The American species, black walnut (*J. nigra* L.), was noted to have injurious effects on other plants during the latter part of the nineteenth century. While some scientists consider it the most notorious of allelopathic trees, others have questioned the allelopathic nature due to the contradictory claims of its impact on other species. Despite anecdotal and experimental evidence on black walnut allelopathy and its potential to negatively impact the growth of other species, a growing body of literature suggests it as a popular tree species for intercropping systems. The objective of this paper is to examine the nature of black walnut allelopathy with particular reference to intercropping systems. We will also examine recent advances in management strategies that have proven to alleviate allelopathic interference in such systems where black walnut is a major component species.

16.2 Black Walnut Allelopathy

Initial reports of black walnut allelopathy surfaced when apple trees were killed in orchards in proximity to walnut trees (Galusha 1870; McWhorter, Douglas, Bryant, and Schroeder 1874). About half a century later, further evidence came from experiments in which tomato plants exhibited growth failure when grown in water and soil media supplemented with black walnut bark (Massey 1925). Field observations by Massey (1925) also confirmed toxic effects of black walnut on plants such as tomatoes and alfalfa within the drip line of the trees. Controversy started when Greene (1930) questioned the toxic nature of black walnut. MacDaniels and Muenscher (1941) provided experimental evidence from a 3-year greenhouse study in which they contradicted the results of Massey. Their study showed no significant difference in the growth of tomato plants, alfalfa, and small apple trees when grown with small walnut trees or walnut roots in containers. Tomato plants watered with leachate from ground-up black walnut roots also did not exhibit any signs of toxicity. However, 1 year later, Brown (1942) reported growth inhibition of tomato and alfalfa by black walnut bark and fresh roots. Numerous reports have been published since then documenting the inhibitory effects of black walnut on associated vegetation (e.g. MacDaniels and Pinnow 1976; Rietveld 1983; Ponder and Tadros 1985; Jose and Gillespie 1998b).

It is now well known that a phenolic compound called juglone (5-hydroxy-1,4-napthoquinone) is the causal agent in black walnut allelopathy (Fig. 1). Juglone was isolated and identified from *Juglans* spp. in the latter half of the nineteenth century (Vogel and Reisohauer 1856). However, it was not until 1925 juglone was suggested as the allelotoxin in black walnut allelopathy (Massey 1925). Later, Davis (1928) confirmed this idea when he demonstrated the toxic effects of synthetic juglone on tomato and alfalfa plants.

Juglone is present, mainly in a reduced nontoxic form called hydrojuglone, in all parts of black walnut, such as roots, bark, twigs, leaf parts, buds, pollen, and fruit parts (Lee and Campbell 1969; Cline and Neely 1984), except the sapwood and heartwood (Gupta, Ravindranath, and Seshadri 1972). When exposed to the air hydrojuglone is oxidized to its toxic form (Gries 1943). Small quantities of juglone can also be found in plant tissues along with hydrojuglone and other precursors and byproducts (Willis 2000).

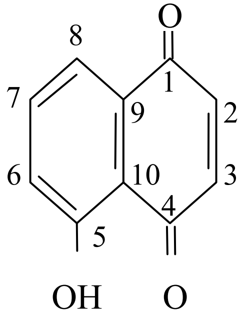

Fig. 1. Structure of juglone (5-hydroxy-1,4-napthoquinone)

16.3 Recent Experimental Evidence for Juglone Phytotoxicity

Several reported studies using known concentrations of juglone (ranging from 10^{-6} to10^{-3} *M*) applied either in hydroponic or soil culture have shown inhibitory effects on survival and growth of several woody plants (Table 1). For example, Rietveld (1983) investigated the sensitivity of several shrubs and trees to juglone in solution culture with juglone concentrations varying from 10^{-3} *M* to 10^{-6} *M*. Although seed germination and radicle elongation were not affected, shoot elongation and dry weight accumulation were affected. Many species were sensitive to juglone concentrations as low as 10^{-6} *M*. Seedlings of all species were severely wilted and eventually killed by 10^{-3} *M* juglone.

In a recent greenhouse study by Ercisli and Turkkal (2005) that investigated the effects of juglone on fruit yield and plant tissue composition of short-day strawberry

Table 1. Percent change in shoot and root dry weights of seedlings grown at different juglone concentrations (*M*) in hydroponic cultures. A negative change means growth was reduced whereas a positive change means growth was enhanced compared to control seedlings

Species	Shoot dry weight (%)			Root dry weight (%)		
	10^{-6}	10^{-5}	10^{-4}	10^{-6}	10^{-5}	10^{-4}
Herbs[a]						
Crimson clover (*Trifolium incanatum*)	–15	–50	–81	11	–50	–78
Crown vetch (*Coronilla varia*)	2	–83	–94	–1	–82	–97
Hairy vetch (*Vicia villosa*)	2	–29	–67	–10	–10	–57
Korean lespedeza (*Lespedeza stipulacea*)	–9	–47	–27	14	–29	–14
Sericea lespedeza (*L. cuneata*)	–30	–72	–92	–6	–63	–88
Shrubs[a]						
Ginnala maple (*Acer ginnala*)	67	64	–35	–35	–28	–83
Siberian peashrub (*Caragana arborescens*)	24	–46	–83	–14	–72	–91
Russian olive (*Elaegnus angustifolia*)	–16	32	–92	8	99	–75
Autumn olive (*E. umbellate*)	–45	–65	–94	–18	–41	–88
Amur honeysuckle (*Lonicera maackii*)	–41	–61	–91	–55	–61	–94
Trees[b]						
White pine (*Pinus strobes*)	–7	–3	–31	–33	–29	–50
Scotch pine (*P. sylvestris*)	–38	0	–63	–50	0	–25
Japanese larch (*Larix leptolepis*)	–36	–14	–71	–20	0	–60
Norway spruce (*Picea abies*)	20	–20	–20	17	–17	–16
Whire oak (*Quercus alba*)	–23	–41	–53	–21	–27	–20
White ash (*Fraxinus americana*)	–20	–58	–83	–7	–31	–71
European black alder (*Alnus glutinosa*)	–33	–86	–94	–26	–87	–94
Yellow poplar (*Liriodendron tulipifera*)	19	8	–72	4	–20	–77
Row crops[c]						
Corn (*Zea mays*)	4	–29	–56	6	–39	–61
Soybean (*Glycine max*)	–11	–37	–33	–9	–48	–56

[a] Seedlings were grown for 4 to 6 weeks; (Rietveld 1983).

[b] Seedlings were grown for 8 to 10 weeks for white pine, scotch pine, Japanese larch, and norway spruce; (Funk, Case, Rietveld, and Phares 1979); the rest of the tree seedlings were grown for 4 to 6 weeks; (Rietveld 1983).

[c] Seedlings were grown for 3 days; (Jose 1997).

(*Fragaria*) cultivars 'Camarosa' and 'Sweet Charlie' in Turkey, the authors reported that plant growth of strawberry was inhibited by juglone. Furthermore, juglone appeared to have a negative impact on plant nutrient uptake. Plant nutrients, including N, K, Ca, Mg, Fe, Mn, Zn, and B were lower in strawberry plant tissues exposed to 1 mM juglone compared to plants in the control treatment (no exposure to juglone) (Ercisli and Turkkal 2005).

Another study conducted in Turkey examined the development of muskmelon (*Cucumis melo* 'Kis kavunu') seeds exposed to 1 mM juglone solution, germinated in petri dishes, and then placed in a growth chamber for 10 days, and reported that the xylem vessel radius of the stem was negatively affected (decreased growth) by the juglone treatment compared to the control treatment (no exposure to juglone) (Terzi, Kocacaliskan, Benlioglu, and Solak 2003, 2004). The authors concluded that this response might by a possible defense mechanism to limit juglone translocation, which in addition, negatively affected water and nutrient translocation, thus decreasing plant growth (Terzi et al. 2003, 2004).

The specific physiological basis for allelochemical effects on plants are difficult to determine since the interruption of one plant process usually affects other processes as well (Boes 1986). Although the physiological mechanisms of juglone on plant species are poorly understood, resent research has begun to shed some light on this subject. For example, Hejl, Einhellig, and Rasmussen (1993) reported respiratory inhibition of soybean mitochondria in response to juglone. They further observed photosynthetic reductions in soybean leaf disks and suggested that changes in normal oxygen uptake by mitochondria would work in concert with impairment of photosynthesis to cause reductions in plant growth. Jose (1997) and Jose and Gillespie (1998b) examined whole plant net photosynthesis, respiration, transpiration and stomatal conductance of hydroponically grown corn and soybean seedlings at various (10^{-4}, 10^{-5}, 10^{-6} M) juglone concentrations. Net photosynthetic rates (P_{net}) were significantly affected by juglone in both species (Fig. 2). The rate of decrease was much greater for soybean than for corn. The lowest concentration did not have any significant effects on P_{net} for either species. Though not significant, a higher P_{net} was observed for corn at the 10^{-5} M compared to the control or 10^{-6} M treatments. This was not seen in soybean where P_{net} was reduced by 72.9% by 10^{-5} M treatment. Relative to the control, the greatest reduction in P_{net} was observed in both corn (67.8%) and soybean (87.1%) at the highest juglone concentration (10^{-4} M). Transpiration rates (E) and stomatal conductance (g) in both species were also significantly affected by juglone (Fig. 2). The trend observed for E and g in response to different juglone levels was similar to that exhibited by P_{net}. However, the effects were less in magnitude as compared to P_{net}.

In order to better understand the mechanisms of the effect of juglone on plant growth, Böhm, Zanardo, Ferrarese, and Ferrarese-Filho (2006) performed a study that examined the cell wall-bound peroxidases and lignin contents of soybean seedling roots exposed to juglone for 24 h. The authors reported that after exposure to juglone (>10 μM), cell wall-bound peroxidase activities, root length, and dry mass decreased, while lignin content increased (Böhm et al. 2006). The consequences of such results could lead to reduced nutrient uptake and plant growth (Böhm et al. 2006) as seen in Ercisli and Turkkal (2005).

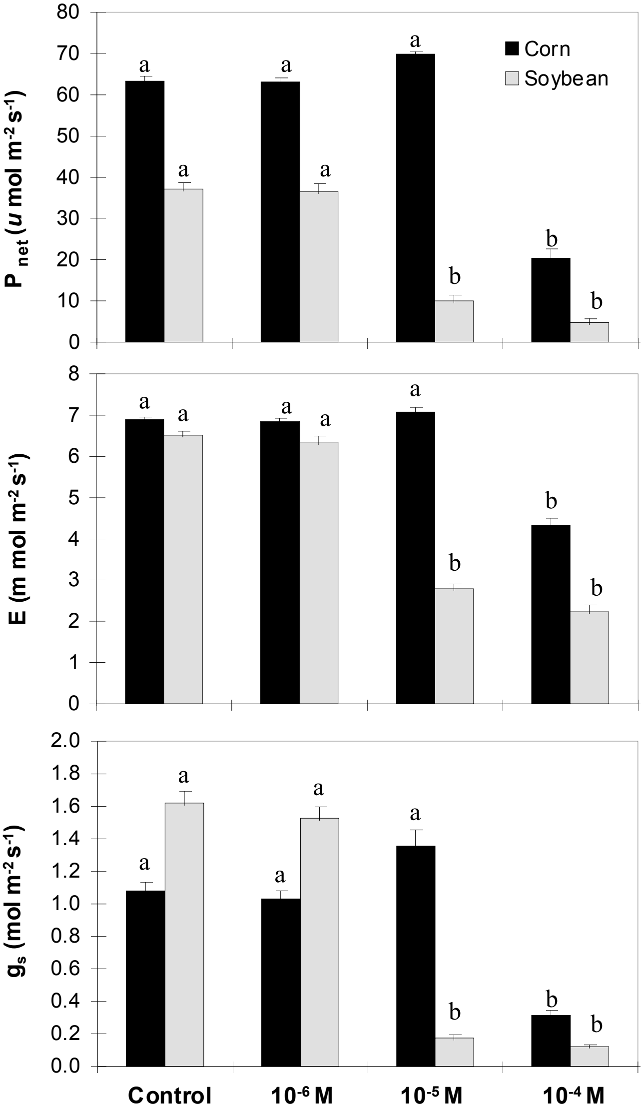

Fig. 2. Influence of juglone on P_{net}, E, and g_s of corn and soybean. Error bars represent one standard error of the mean. Bars with the same letters are not significantly different (Jose and Gillespie 1998b)

Meazza, Scheffler, Tellez, Rimando, Nanayakkara, Khan, Abourashed, Romagni, Duke, and Dayan (2002) showed that a crucial enzyme for plastoquinone synthesis, *p*-hydroxyphenylpyruvate dioxygenase is inhibited by juglone. Others have shown that juglone reduces H^+-ATPase activity, which translates into an inability of root cells to establish a proton gradient, therefore limiting water and solute uptake and eventually decreasing plant growth (Hejl and Koster 2004).

16.4 Why is Black Walnut Popular?

As mentioned earlier, despite the allelopathic nature of black walnut, it is still a popular tree species for intercropping systems in the United States, owing to its highly valuable timber and nuts. It is found throughout eastern United States hardwood forests, most commonly growing as scattered individual trees or in small groves (Williams 1990). Black walnut can grow on a variety of sites, but grows best on well-drained, moist, fertile soils (Williams 1990). The highly coveted wood from quality black walnut trees is most commonly used for veneer. The stumpage prices for black walnut veneer logs harvested in the Midwestern United States are often double that of other eastern hardwood veneer species such as white oak (*Quercus alba* L), red oak (*Quercus rubra* L.), and sugar maple (*Acer saccharum* Marsh.) (Hoover and Preston 2006).

In addition to its value as a timber species, black walnut is also an important nut producing species. Over 11 million kilograms of wild black walnuts are collected each year, creating a $2.5 million industry for nut collectors (Chamberlain, Bush, and Hammett 1998). Black walnut nutmeat is used for food for human consumption while the shells have a multiple industrial uses (Das, Shillington, and Hammett 2001). Potential income from wood and nut production makes black walnut a popular plantation species. In plantations managed for nut production and timber, financial returns from the land can be gained as soon as the trees are old enough to produce nuts (about 15–20 years after planting), therefore offsetting some of the risks and costs of long-term timber production (Garrett, Jones, Kurtz, and Slusher 1991).

16.5 Black Walnut Intercropping

16.5.1 Alley Cropping

Even with early financial returns from nut harvesting, establishment of tree plantations can be expensive. Alley cropping is the practice of planting cultivated crops between widely spaced tree rows. One of the major benefits of intercropping is that it can be used to offset the costs of establishing tree plantations. Economic evaluations of black walnut intercropped systems have concluded that intercropping creates a greater financial return than land managed for timber production alone (Garrett et al. 1991). Furthermore, where site quality is high, intercropping may create greater returns than traditional agriculture (Campbell, Lottes, and Dawson 1991).

Black walnut is an ideal species for intercropping because it grows rapidly, produces a high quality timber product, and has a nut-producing capacity. In addition, there are many physiological aspects as well that make black walnut desirable for intercropping. In the spring, black walnut is one of the last species to leaf out, and in the fall one of the first to drop its leaves, reducing competition for sunlight. Even with full foliage, black walnut still allows about 50% of full sunlight to reach the understory (Moss 1964), and sunlight was not considered to be the primary limiting factor of grain yields in young black walnut and corn alley cropping system in southern Indiana (Gillespie, Jose, Mengel, Hoover, Pope, Seifert, Behle, Stall, and Benjamin 2000). Black walnut also has an ideal root system for intercropping. The species produces a large taproot and has a deep rooting system, leaving the uppermost portions (<10 cm) of the soil horizon available for root growth of companion species (Yen, Pham, Cox, and Garret 1978). However, even with the relatively deep rooting system of black walnut, Jose, Gillespie, Seifert, and Biehle (2000), reported that competition for water that decreased grain yields in a young southern Indiana black walnut and corn alley cropping system.

In North American, black walnut is most commonly intercropped with corn (*Zea mays* L.) and soybeans (*Glycine max* L. Merr.) (Garrett et al. 1991; Gordon and Williams 1991). Additional row crops that are sometimes planted include winter wheat (*Triticum aestivum* L.), barely (*Hordeum vulgare* L.) and milo (*Sorghum bicolor* (L.) Moench) (Garrett and Harper 1999). Yield reductions of associated species in alley cropping systems have been reported. For example, Gillespie et al. (2000) reported a 39% yield reduction in corn when intercropped with black walnut trees. Although competition for water was suspected as the primary reason for the observed yield reduction (Jose et al. 2000), allelopathy was not ruled out either (Jose and Gillespie 1998a). Significant quantity of juglone was observed in soil under black walnut, which prompted the authors to conduct greenhouse experiments to examine the sensitivity of corn and soybean to juglone. Three different concentrations of juglone (10^{-4}, 10^{-5}, and 10^{-6} *M*) along with a control were applied to corn and soybean in solution culture. Within 3 days, juglone exhibited significant inhibitory effects on shoot and root relative growth rates. In general, soybean was found to be more sensitive to juglone than corn. Root relative growth rate was the most inhibited variable for both species, and reductions of 86.5% and 99% were observed in corn and soybean, respectively, at 10^{-4} *M* juglone concentration. Actual juglone concentrations in the field may not be as high as 10^{-4} *M*. However, concentrations as high as 2×10^{-5} *M* have been reported under field conditions (Ponder and Tadros 1985; Jose and Gillespie 1998a).

In a recent greenhouse study, Napolitano and Jose (unpublished data) observed significant reductions in growth of cotton (*Gossypium* spp.) and Peanut (*Arachis hypogaea* L.) when exposed to juglone in hydroponic cultures. These are two other species with potential for alley cropping with black walnut trees.

16.5.2 Silvopastoral Systems

Silvopasture is another system that can be used to offset the costs of establishing black walnut plantations. In silvopasture, livestock is introduced into the system and the forage crops are grazed. Garrett et al. (1991) calculated the highest internal rate of return for the black walnut management regimes that included silvopasture and similar results have been observed in other silvopastoral systems as well (Sharrow, Carlosn, Emmingham, and Lavender 1996). One drawback of silvopasture in young hardwood systems is the potential for livestock to damage young seedlings (Lehmkuhler, Kerley, Garrett, Cutter, and McGraw 1999; Garrett and Kurtz 1983) and initial costs of fence construction (and subsequent maintenance). This makes the implementation of this system potentially expensive, however, if livestock is not introduced into the system until the trees are large enough to withstand damage (about 15 years depending on site quality) then fencing is unnecessary (Garrett and Kurtz 1983). Furthermore, because forage crops tend to fare better under shaded conditions than corn and soybeans, it is possible to continue silvopasture when trees have matured and cultivation of row crops is not possible (Garrett and Harper 1999).

Forage crops, such as clover species (*Trifolium* spp.), orchardgrass (*Dactylis glomerata* L.), and red fescue (*Festuca rubra* L.) can also be planted in alleyways to be harvested as a hay crop in silvopastoral systems (Delate, Holzmueller, Mize, Frederick, and Brummer 2005; Alley, Garrett, McGraw, and Blanche 1999). Some forage crops yield more under lightly shaded conditions and nutritional content is superior when compared to forage grown in open systems (Kallenbach, Kerley, and Bishop-Hurley 2006; Garrett and Kurtz 1983). Furthermore, certain forage crops, particularly cool season legumes, are considered less competitive than warm-season grasses and can act as living mulches for trees that can suppress weed growth (Delate et al. 2005; Alley et al. 1999). In a study conducted in central Iowa, tree growth for two hardwood species, red oak (*Quercus rubra* L.) and black walnut, were compared using forage crop combinations (oats/red clover, oats/red clover/red fescue, and oats/red clover/orchardgrass) and herbicides as weed control treatments (plus a control) on an upland and bottomland site.

After 4 years of growth, black walnut seedlings growing in the oats/red clover/red fescue treatment were comparable in height to seedlings grown in the herbicide treatment and significantly higher than the control treatment in both the upland and bottomland sites (Fig. 3; Holzmueller 2002), indicating that this was an ideal companion crop mixture to plant with black walnut. In addition, 2 years after establishment of the experiment there was no difference in forage yield and quality harvested between the two tree species (Delate et al. 2005). This perhaps illustrates the fact that black walnut allelopathy may not be a factor for forage growth in young plantations.

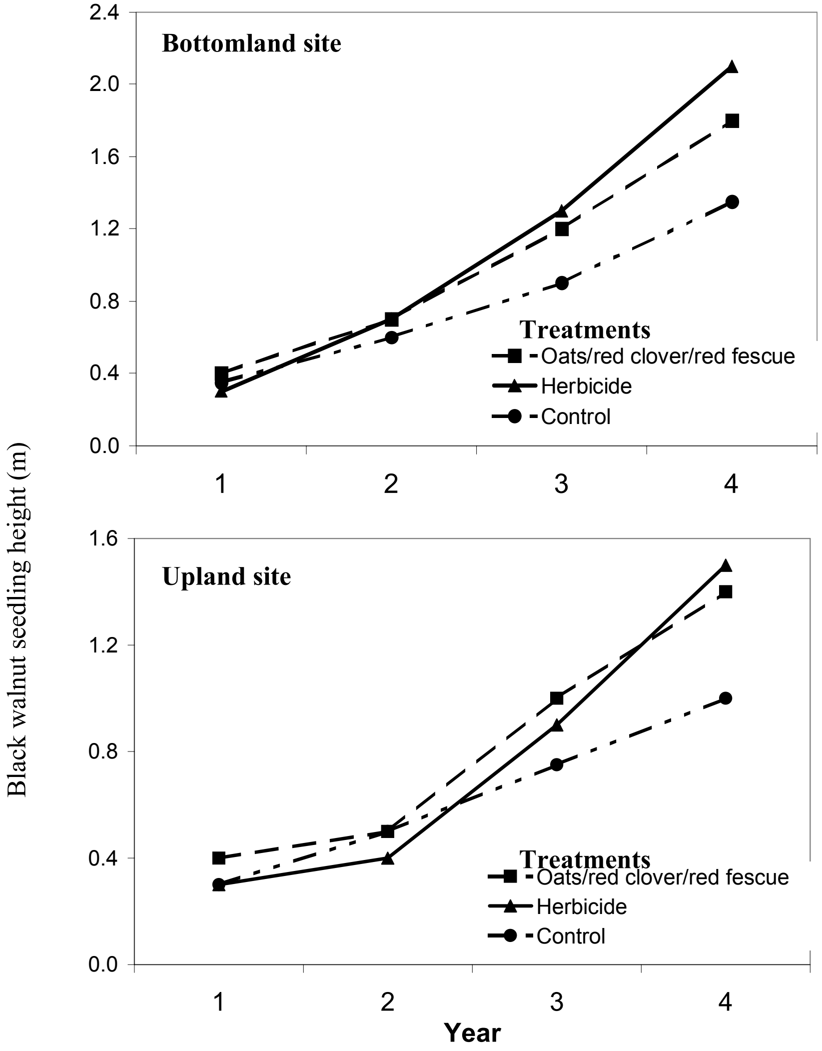

Fig. 3. Seedling height for black walnut trees established in an intercropped system on two sites (upland and bottomland) under various weed control measures: oats/red clover/red fescue (living mulch), herbicides, and a control (no treatment). After four growing seasons, tree height for seedlings in the oats/red clover/red fescue treatment were comparable with the herbicide treatment, while both of these treatments were significantly higher than the control on both sites (Holzmueller 2002)

16.5.3 Mixed Species Plantations

Most of the field evidence for black walnut allelopathy has come from mixed species tree plantations involving black walnut. For example, Perry (1932) reported the death of a white pine (*Pinus strobus* L.) stand in response to black walnut invasion or proximity. Another incident of white pine mortality took place at the Cunningham Experimental forest owned by Purdue University in Indiana, USA. (W. Beineke, written communication). A white pine plantation planted in 1948 was invaded by black walnut. By 1962, the white pines were losing vigor and dying and by 1971 all the white pines had been killed.

Other species such as white birch (Gabriel 1975), American elm (*Ulmus americana* L.), and ash (*Fraxinus pennsylvanica* Marsh.) (Bratton 1974) have also been found to be sensitive when interplanted with black walnut trees. These species were either declining or killed in the vicinity of black walnut trees. A recent study reported death of alder trees interplanted with black walnut (Bohanek and Groninger 2005). Alder mortality began to increase dramatically during the seventh growing season and was nearly complete following the nineteenth growing season. Similar results were reported by Ponder and Tadros (1985). Althen (1968) reported that red oak (*Quercus rubra* L.) interplanted with black walnut appeared healthy and grew normally, while red pine (*Pinus resinosa* Ait.) began to die after 15 years. Black walnut toxicity to red pine has also been reported by Brooks (1951) under field conditions.

16.6 Management Implications

An allelopathic role for juglone would require its release from tissues and accumulation in the soil in sufficient quantities to inhibit the growth of other plants. Juglone may enter the soil through processes such as root exudation, litter decay, and throughfall. Juglone produced in the leaves is translocated to other plant parts and the roots act as a major sink for juglone and its precursors. But, how much of the juglone is released into the soil? How long will it take to accumulate enough juglone in the soil to cause inhibitory effect on other plants? These questions are extremely important in managing intercropping systems involving black walnut.

The accumulation and persistence of juglone in soil will depend on local site and soil conditions. Although early reports suggested juglone's presence only in the immediate vicinity of roots and its lack of persistence (Massey 1925; Pirone 1938), recent reports indicate a gradient of juglone levels with respect to depth and/or distance from black walnut trees (Ponder and Tadros 1985; Ponder 1987). In a 14-year-old black walnut plantation, Ponder and Tadros (1985) reported juglone concentrations (at a depth of 0–8 cm) ranging from 1.85 to 3.95 $\mu g\ g^{-1}$ soil at a distance of 0.9 m from the trees. However, sampling at a distance of 1.8 m resulted in lower concentrations ranging from 0.70 to 1.55 $\mu g\ g^{-1}$ soil. In another study, De Scisciolo, Leopold, and Walton (1990) reported juglone concentrations of up to 1.88 $\mu g\ g^{-1}$ soil in a 73-year-old black walnut plantation mixed with other hardwoods (within a distance of 0.5 to 1.0 m from tree base and to a depth of 10 cm). Jose and Gillespie (1998a) measured soil juglone levels in a 10-year-old black walnut plantation and reported significant decrease in juglone levels with distance from trees (Fig. 4). When the distance increased to 4.25 m from the tree row, juglone concentration decreased as much as 80% (0.31 μg g^{-1} soil) as compared to the within-tree row concentration (1.63 μg g^{-1} soil).

It appears that when sensitive plant species are exposed to juglone, water and nutrient uptake is disrupted, leading to a reduction in plant growth and crop yields. In field conditions this problem may become exacerbated under drought conditions where water is a limiting factor to plant growth and yield (Jose et al. 2000). In alley cropping or silvopastoral systems, management techniques to counter this problem, such as trenching or separation of the root systems of the crops from that of the trees, may be required in order to maximize crop production. For example, Jose et al. (2000) demonstrated that by separating the root systems black walnut and corn using a polyethylene barrier, crop yield became similar to that of monoculture. These authors further showed that the juglone concentration in the soil was negligible beyond the polyethylene barrier (Fig. 5). Juglone concentration beyond the root barrier decreased to trace levels of 0.08 and 0.01 μg g^{-1} soil (at a distance of 2.45 and 4.25 m, respectively) in the 'root barrier' treatment compared to 0.42 and 0.32 μg g^{-1} soil in the 'no barrier' control treatment. However, juglone levels were significantly higher at 0 and 0.9 m (2.53 and 2.51 μg g^{-1} soil) in the 'root barrier' than in 'no barrier' (1.63 and 1.44 μg g^{-1} soil) treatment. This result also supports the idea that roots produce most of the juglone present in the soil. The higher concentration of juglone within the root barrier (i.e., at 0 and 0.9 m) may be due to enhanced root proliferation following root pruning as shown by Jose et al. (2000). Confining the root mass within a restricted volume of soil (by installing the polyethylene barrier) could also cause juglone to build up in greater concentrations as rooting density increases. Beyond the barrier, one would expect either no roots or only a negligible rooting density (Jose and Gillespie 1998a) and that was reflected in the significantly lower juglone levels observed.

While polyethylene barriers may prove to be effective, is it always a viable option for landowners? Are there any cheaper alternatives to installing barriers? Trenching or deep disking a few meters away from the tree base may be as effective as polyethylene barriers if done regularly. If practiced while trees are young, repeated disking or trenching may force tree roots grow deeper. Early results from a trial at the University of Florida point to the possibility of training trees roots to grow deeper and occupy lower soil horizons, therefore decreasing competition for moisture and nutrients and perhaps reducing the effects of juglone (Jose, unpublished data). If tree roots occupy a deeper rooting zone compared to the crop roots, they could act as a safety-net and 'capture' nutrients and water that leach down the crop rooting zone (Allen, Jose, Nair, Brecke, and Nkedi-Kizza 2004). This belowground niche separation will reduce direct competition for nutrients and water between black walnut and crop species while alleviating the allelopathic interference resulting from juglone.

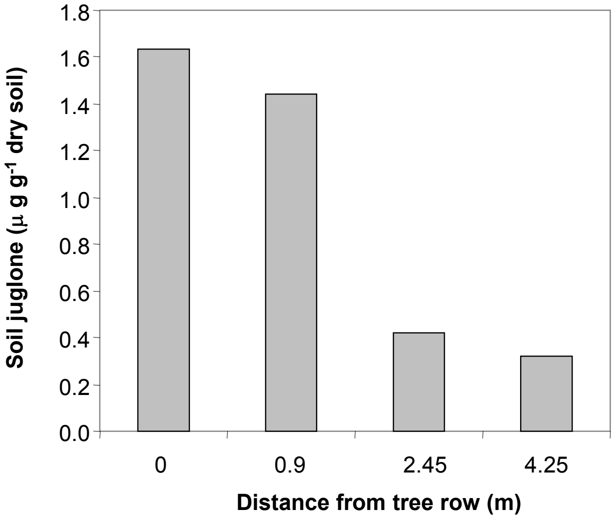

Fig. 4. Soil juglone concentration as influenced by distance from tree row (Jose and Gillespie 1998a; Jose 2002)

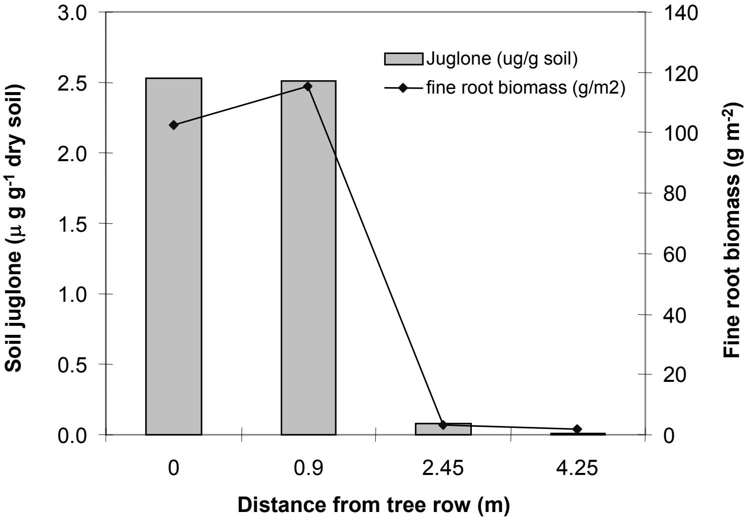

Fig. 5. Soil juglone concentration as influenced by polyethylene root barrier inserted at a distance of 1.2 m from tree rows. The barrier prevented tree roots from growing beyond 1.2 m. Soil juglone concentration was also negligible beyond the root barrier (Jose 1997; Jose 2002)

Species selection can also play a major role in the success of intercropping systems involving black walnut. Species vary widely in their sensitivity to juglone. For instance, multiple studies have indicated that soybeans are more susceptible to juglone than corn (Jose and Gillespie 1998b; Hejl and Koster 2004). Reasons for this difference could be explained by the work conducted by Sparla, Preger, Pupillo, and Trost (1999) who reported that corn produces a flavoreductase that potentially detoxifies juglone, an idea that is supported by the work conducted by Hejl and Koster (2004). Kocacaliskan and Terzi (2001) reported that seedling growth of 11 different species was not uniform when exposed to juglone. Tomato (*L. esculentum* 'Rio Grande'), cucumber (*Cucumis sativus* 'Cengelköy'), alfalfa (*Medicago sativa* 'Yerli') where some of the species where seedling growth reduction was highly significant when exposed to juglone, while corn, wheat (*Triticum vulgare* 'Gönen'), and barely (*Hordeum vulgare* 'Tokak') were species where seedling growth reduction was only slightly significant when exposed to juglone (Kocacaliskan and Terzi 2001).

It is also possible to avoid the allelopathic effects of juglone if companion species are planted and managed while black walnut trees are young. Based on the published literature it is safe to assume that it takes about 10 years for juglone to reach high enough levels in the soil to begin to affect other species. Perhaps this window of opportunity could be made use of in growing agronomic crops or pasture or short rotation tree species during the early establishment phase of a black walnut plantation.

16.6 Conclusions

Despite its controversial role as an allelopathic species, black walnut is a highly sought after tree species for intercropping systems in the United States. The highly valuable timber and potential for nut production are two major reasons for its popularity; however, other characteristics such as open canopy and rapid growth also make it suitable for intercropping. While many of the species interplanted with black walnut in alley cropping, silvopasture and mixed species plantations are sensitive to juglone and experience growth reductions and mortality, recent advances in management techniques have proven to be effective in alleviating the negative effects to a large extent. Management techniques include the use of polyethylene root barriers that separate the root systems of the trees from that of the crops of other companion species, trenching or disking to train the tree roots grow deeper, and planting and managing the companion species during the early establishment phase of black walnut trees when soil juglone levels are negligible. The interest in black walnut intercropping seems to be growing. While allelopathic interference is a reality in such systems, management intervention may help sustain the production potential so that they become an economically viable option for landowners.

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17. Plant Growth Promoting Rhizobacteria and Mycorrhizal Fungi in Sustainable Agriculture and Forestry

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Abstract. Plant-growth promoting rhizobacteria (PGPR) encourage plant growth by producing growth regulators, facilitating nutrient uptake, accelerating mineralization, reducing plant stress, stimulating nodulation, providing nitrogen fixation, promoting mycorrhizal fungi, suppressing plant diseases, and functioning as nematicides and insecticides. Many of the PGPR are fluorescent pseudomonads (*Pseudomonas fluorescens*), but other bacteria (*Bacillus* sp., *Azotobacter* sp., *Acetobacter* sp., *Azospirillum* sp.) are known as well. Many of these organisms have been formulated into biofertilizers and are commercially available. However, there is a disconnect between the demonstration of the growth-promoting activity of these organisms in laboratory and field studies versus their use in commercial production. The reason for this is two-fold. First, there have been inconsistent results between experimental studies and practical field applications where the growth-promoting activities of the rhizobacteria are masked by other environmental and management factors. Second, there is a lack of technology transfer and education, thus limiting the farmers' use of biofertilizers. Here we review the role of rhizobacteria stimulating plant growth and their use as biofertilizers; indicate that the use of biofertilizers may be of more benefit in unproductive and stressful environments; and recommend that commercially available biofertilizers be evaluated in standardized field tests.

17.1 Introduction

As stated recently by An (2005) “Allelopathy arises from the release of chemicals by one plant species that affect other species in the vicinity, *usually* to their detriment.” (We have added the emphasis on “usually.”) This is a generally accepted definition of allelopathy. Although Molisch (1937) defined allelopathy to include both beneficial and harmful effects of one plant or microorganism on another, the majority of allelopathy studies are concerned with inhibitory effects. This may in part be due to interest in using allelochemicals as alternatives for synthetic pesticides. Or as pointed out earlier, stimulatory effects are often not as spectacular as inhibitory effects and have been generally ignored (Mallik and Williams 2005). However, there are reports of stimulation of plants by other plants and microorganisms, and visa-versa, which we reviewed earlier (Mallik and Williams 2005). Here we review allelopathic stimulation focusing on rhizosphere microorganisms, and specifically the role of rhizobacteria as biofertilizers.

As the plant root system develops, organic compounds (root exudates) are released into the soil. Root exudates may include passive leakage of low molecular weight compounds (sugars and amino acids), as well as active secretion of high molecular weight compounds across cell membranes (polysaccharides, proteins, fatty and other organic acids, phytohormones and enzymes). The composition of root exudates depends on plant species, growing conditions, plant growth stage, and rooting medium. Exuded compounds are used as nutrients by the numerous microorganisms contained in the rhizosphere, and in turn the compounds released by the microorganisms, either as exudates or metabolic products, affect the quantity and quality of compounds released by the root system (Bolton, Fredrickson and Elliot 1993). The system is highly dynamic and suggests a degree of co-evolution between rhizobacteria and their associated plants (Bolton et al. 1993).

Plant growth-promoting rhizobacteria, a term first used by Kloepper and Schroth (1978), can directly or indirectly promote plant growth (Fig. 1). Some PGPRs may promote plant growth by producing growth regulators that stimulate other beneficial rhizobacteria, stimulate the plant directly, aid in nodulation, or indirectly stimulate nodulation (Fig. 1, 1a–1d). Other PGPRs accelerate mineralization and uptake of certain nutrients (Fe, P, Mn, Zn and Cu) (Tinker 1984) (Fig. 1, 2b). Growth promotion can also occur indirectly when PGPRs function as biocontrol agents of soil-borne plant pathogens and weeds, as promoters of mycorrhizal fungi, provide biological nitrogen fixation (biofertilizer) (Fig. 1, 1e and 2a), or by reducing the negative effect of deleterious rhizobacteria (DRB) (Fig. 1, 3b). However, the major function of PGPR is through the suppression of plant pathogens by releasing antibiotics, cyanide, and enzymes (Kloepper, 1993) (Fig. 1, 3a–3b). Since the rhizosphere is a complex mixture of microorganisms and their numerous interactions, the resulting stimulation of plant growth is probably multifaceted in many cases.

The interest in developing plant growth-promoting rhizobacteria (PGPRs) as crop additives has increased over the past 20 years. What has stimulated the interest in this area? First, the public perception of environmental pollution resulting from the use of synthetic chemicals in agriculture has led to the realization that present agricultural practices should shift from the use of large inputs of fertilizers and pesticides to more environmentally-friendly production practices. Second, if we are to achieve sustainable agriculture, particularly in areas that are resource limited, we must find methods to sustain crop yield and reduce production costs. Beneficial rhizobacteria have potential as part of an overall management system to reduce the use of synthetic compounds and fertilizer, and provide a sustainable agriculture.

This review provides examples of the growth promoting activities of allelopathic rhizobacteria. References cited draw attention to allelopathic stimulation, with the view to exploit the phenomenon where feasible in agriculture and biological research. An extensive review of the allelopathic literature covering this topic is not intended. Production of growth regulators will not be discussed and the reader is referred to Arshad and Frankenberger (1993, 1998), Zahir, Arshad and Frankenberger (2004) and Mallik and Williams (2005).

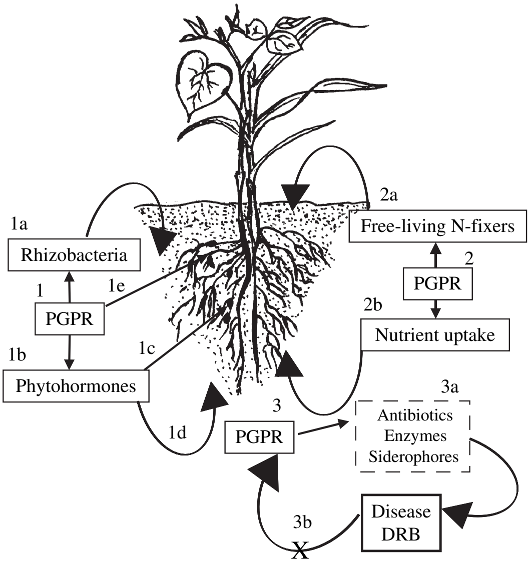

Fig. 1. Possible indirect and direct pathways PGPRs may influence plant growth

17.2 Rhizobacterial Effects on Plant Growth

17.2.1 Plant Disease Control

PGPR-induced systemic disease resistance (ISR) was first reported by Scheffer (1983) when he discovered that prior inoculation of elm trees with four fluorescent pseudomonad strains led to significant reduction in foliar symptoms of Dutch elm disease caused by the fungal pathogen *Ophiostoma ulmi*. Since then, this immunization, or induction of systemic disease resistance, has been reported in a wide variety of plants (Table 1). The inhibition of a phytopathogen by a PGPR can occur via release of a toxic compound, antibiotic or enzyme; or through rapid colonization of the root zone blocking the phytopathogen or DRB development.

While the production of HCN by *Pseudomonas fluorescens* was cited in the suppression of *Thielaviopsis basicola* in tobacco (Keel, Voisard, Berling, Kahr and Defago 1989), suppression of DRB in sugar beet was due to the large population density of the introduced PGPRs (Suslow and Schroth 1982). Potato seed tubers treated with a cell suspension of three fluorescent *Pseudomonas* isolates increased subsequent plant growth and yield, and in this case the authors concluded that PGPR isolates produced a significant amount of siderophores resulting in suppression of DRB by iron deprivation (Geels and Schippers 1983).

PGPR-induced systemic disease resistance may result from biochemical responses in the host plant. Increased phytoalexin levels were reported in carnation inoculated with *Pseudomonas* sp. (van Peer, Niemann and Schippers 1991), while increased levels of protein were found in bean and tomato following seed treatment with a PGPR (Hynes and Lazarovits 1989). In other studies, increased peroxidase activity localized on the root surface (Albert and Anderson 1987) and lignification of stems/leaves in bean (Anderson and Guerra 1985) and potato (Frommel, Nowak and Lazarovits 1991), after colonization by an introduced PGPR, were related to suppression of the phytopathogen.

Table 1. Examples of growth-promoting rhizobacteria (PGPR) used in disease control

Crop	PGPR	Result	Reference
Carnation	*Pseudomonas* sp.	Induced resistance to *Fusarium oxysporum*	Duijff et al. 1994
Cucumber	Unknown	Induced resistance to mosaic viruses	Raupach et al. 1996
Cucumber	*Pseudomonas putida* *Serratia marcencens*	Induced resistance to *Fusarium* sp.	Liu et al. 1996
Cucumber	Unknown	Induced resistance to Angular leaf spot	Liu et al. 1995
Bean	*Pseudomonas* sp.	Reduced leaf lesions of *Pseudomonas syringae*	Alstrom 1991
Sugar beet	*Pseudomonas* sp.	General protection against pathogens	Suslow and Schroth 1982
Potato	*Pseudomonas* sp.	Suppression of deleterious rhizobacteria	Geels and Schippers 1983
Cotton	*Pseudomonas fluorescens*	Induced resistance to *P. ultimum* and *Rhizoctonia solani*	Howell and Stipanovic 1979, 1980
Wheat	*Pseudomonas fluorescens*	Resistance to *Gaeumannomyces* sp.	Weller and Cook 1983, 1986

Agrobacterium, *Bacillus*, *Burkholderia*, *Erwinia* and *Pseudomonas* species are known antibiotic producers (Kloepper 1994), and 90% of the antibiotic producers also produce siderophores. An antibiotic producing wild strain of *P. fluorescens,* genetically altered to over-produce pyoluteorin and 2,4 diacetylphloroglucinol, effectively protected cucumber plants against *Pythium ultimum* infection (Schneider and Ullrich 1994). Damping-off caused by *P. ultimum* and/or *Rhizoctonia solani* was controlled in cotton by treating seed with *P. fluorescens* pf-5 that produced the antibiotics pyoluteorin and pyrrolnitrin (Howell and Stipanovic 1979, 1980). In naturally infested fields, wheat take-all disease (*Gauemannomyces graminis*) was suppressed in spring and winter wheat by inoculating the seed with *P. fluorescens*. In this case control was linked to the increased level of 2,4-diacetylphloroglucinol or phenazine-1-carboxylate produced by the pseudomonads (Weller and Cook 1983, 1986).

Fusaric acid is a common compound in *Fusarium* infection. Several PGPRs (*P. cepacia = Burkholderia cepacia*, *P. solanacearum*) are capable of hydrolyzing fusaric acid, which controls the *Fusarium* infection (Toydoa, Hashimoto, Utsumi, Kobayashi and Ouchi 1988). Lim, Kim and Kim (1991) isolated a strain of *P. stutzeri* that produces two enzymes (chitinase and laminarinase) that lyse *Fusarium* mycelium preventing the fungus from causing root rot in several plant species. Fridlender, Inbar and Chet (1993) isolated the enzyme β-1,3 glucanase from a strain of *P. cepacia* that injures fungal mycelia and reduces plant damage caused by *Rhizoctonia solani*, *Sclerotium rolfsii* and *P. ultimum*.

These examples show direct effects, but suppression of plant diseases may be indirect. For example, suppression of *P. ultimum* on sugar beet is probably due to the ability of the introduced pseudomonad to utilize sugar beet exudates to produce compounds inhibitory to the pathogen (Stephens 1994). Part of this suppression may also be due to a reduction in the nutrients available for the pathogen. Ferric ion (Fe^{3+}), the predominant form of iron, is barely soluble. Since available iron is too low to directly support bacterial growth, soil microorganisms secrete low molecular weight siderophores that bind ferric ions and transport them back to the cell membrane, which forms an appropriate receptor compound and makes the iron available for microbial growth (Volk and Wheeler 1980). This process binds most of the available iron in the rhizosphere and prevents the pathogens from developing (O'Sullivan and O'Gara 1992; Tate 2000).

Most plants can grow at low concentrations of available iron, and several plants can bind iron with their own siderophores (Wang, Brown, Crowley and Szaniszlo 1993). Fourteen *Burkholderia cepacia* strains were isolated from a corn rhizosphere and tested for siderophore production and antibiosis against two species of *Fusarium* corn-root pathogen (Bevivino, Sarrocco, Dalmastri, Tabacchioni, Cantale and Chiarini 1998). Hydroxamate-like and thiazole-like siderophores were detected in the culture medium of each strain. Several of the isolates inhibited in vitro growth of *F. moniliforme* and *F. proliferatum*. Antibiosis was more evident in an iron-deficient medium, which suggested the Fe^{3+} deficiency might have enhanced siderophores production and antibiosis (Bevivino et al. 1998). Siderophore production is an effective mechanism in disease suppression. Although the producing agent is affected by several biotic factors (the pathogen, PGPR, type of siderophores produced and the target plant), the use of siderophore-producing PGPR as biocontrol agents for plant pathogens has potential and should be evaluated further.

Many of the examples provided involved crop plants, but PGPRs are also used in forestry to inhibit pathogens. Fungal root disease causes considerable seedling loss in conifer nurseries and reduces seedling survival and growth in reforestation sites. *Burkholderia cepacia* (strain RAL3) and *P. fluorescens* (strain 64-3) reduced (7–42%) *Fusarium oxysporum* root disease in Douglas fir, improved white spruce seedlings survival when planted in soil inoculated with *Fusarium* sp. and *Pythium* sp. in a nursery, and increased (19–23%) survival of bare-root white spruce seedlings planted on a reforestation site as compared to the control (Reddy, Funk, Covert, He and Pedersen 1997). Further discussion on forestry application is given in Section 7.3 "Biofertilizers in Production."

17.2.2 Promotion of Symbiotic Biological Fixation

A few PGPRs have been used to stimulate nodule formation, growth and number, and nitrogen fixation in several legumes. Of 17 *P. fluorescens* and *P. putida* isolated from the root surface of soybean (Polonenko, Scher, Kloepper, Singelton, Laliberte and Zaleska 1987) nine isolates increased nodule weight, while three isolates increased both nodule number and weight. Several strains also increased soybean shoot and root dry weight, but these effects were not associated with an increase in nodule number or nodule weight. In a field study, nine PGPR strains (seven pseudomonads and two *Serratia* sp.) were tested for their effects on nitrogen fixation in lentil and pea inoculated with *Rhizobium leguminosarum* (Chanway, Hynes and Nelson 1989). Pea growth was unaffected; but growth, nodulation and acetylene reduction in lentil were significantly increased by two *P. putida* strains. These results, verified in the laboratory, suggest that these PGPR strains might be useful as inoculants for lentil, depending on the cultivar and growing conditions (Chanway et al. 1989).

Nodulation and N_2-fixation of soybean plants are hampered by cool soil temperatures. Zhang, Dashti, Hynes and Smith (1996) demonstrated that co-inoculation of soybean a PGPR and *Bradyrhizobium japonicum* increased nodulation at cooler soil temperatures. Bai, Zhou and Smith (2003) isolated three *Bacillus* strains from a nodule of field grown soybeans that displayed growth promoting activity. Soybean was inoculated with these strains and *Bradyrhizobium japonicum* and the plants grown under controlled conditions and in the field. Soybean co-inoculation with *Bacillus thuringiensis* NEB 17 provided the most consistent results and the largest increase in total plant biomass, root and shoot weight, nodulation (total number and weight), total nitrogen and grain yield.

These studies, particularly the last two, indicate that PGPRs and rhizobia co-inoculation could improve nodule formation and N_2-fixation, and that co-inoculation may be of a greater value under stress conditions (temperature, salinity or moisture). However, further screening of PGPR strains and testing under various field conditions needs to be done.

17.2.3 Associative Diazotrophs

Associative diazotrophs have gained importance recently as a source of nitrogen for crop production. Beneficial effects of associative diazotrophs (e.g. *Azotobacter*) have been investigated in Europe, particularly in Russia and, since the report of their discovery in grass roots in the late 1970s, diazotrophs (e.g. *Acetobacter*) have been widely studied. Associative diazotrophs include *Azotobacter, Azospirillum, Azomonas, Herbaspirillum, Spirillum, Acetobacter, Beijarinckia, Azoarcus, Burkholderia, Clostridium* and several genera belonging to the Enterobacteriaceae. Here we provide a few examples.

Azospirilla, micro-aerophyllic, heterotrophic diazotrophs have been investigated as possible nitrogen fixing bacteria for grasses since their discovery on the roots of tropical grasses (Day and Dobereiner 1976). Soil application or seed inoculation of *Azospirillum lipoferum* resulted in a 22% increase in rice grain yield in field experiments (Balandreau 2002) and enhanced P and ammonia uptake by the plants (Murty and Ladha 1988), while a 30% yield increase was reported for wheat inoculated with *A. brasilense* (Okon and Labandera-Gonzales 1994). Although these yield increases can be attributed in part to increased nitrogen availability, it was estimated using ^{15}N dilution technique measurements that the *Azospirillum*-root association in grasses and cereals contributed only 1–10 kg N/ha (Kapulnik, Feldman, Okon and Henis 1985). In other work, 12% of the nitrogen accumulated by corn was contributed by *Azospirillum* (Rennie 1980). Some of the yield increases may be due to indirect effects of *Azospirillum* sp. *Azospirillum* inoculation has enhanced root and root hair growth, resulting in significant increase of nitrogen (Fayez and Daw 1987) and mineral uptake (Lin, Okon and Hardy 1983), as well as the production of antifungal and antibacterial compounds, growth regulators and siderophores by the inoculated plants (Pandey and Kumar 1989; Fallik, Sarig and Okon 1994; Okon and Labandera-Gonzales 1994). Based on 20 years of field application data, Okon and Labandera-Gonzales (1994) concluded that *Azospirillum* can increase crop growth and yield by 5 to 30% depending on soil and climatic conditions.

*Azotobacter*s are aerobic heterotrophic associative N_2-fixers, provided an adequate supply of reduced carbon compounds and low oxygen pressure favorable for nitrogenase activity are available. *A. chroococcum* and *A. vinelandii* have been used widely in various studies, and the genus has been reported to increase the yield in rice (Yanni and Abd El-Fattah 1999), and replaced up to 50% of the inorganic nitrogen fertilizer requirements for wheat (Hegazi, Faiz, Amin, Hamza, Abbas, Youssef and Monib 1998). *A. paspali* was first isolated from a grass, *Paspalum notatum* (Dobereiner and Pedrosa 1987). Boddey, Chalk, Victoria, Matsui and Dobereiner (1983) calculated that 11% of the nitrogen accumulated by the grass was contributed by *A. paspali*.

Acetobacter (Gluconacetobacter) diazotrophicus is an endophytic, acid tolerant biological nitrogen fixer (BNF). Boddey, Urquiaga, Ries and Dobereiner (1991) calculated, based on ^{15}N dilution studies, that 60–80% of sugar cane plant nitrogen (equivalent to 200 kg N/ha) is derived from BNF, and that *Acetobacter diazotrophicus* was the principal contributor. Because of this, seedling inoculation with an effective *Acetobacter* strain has become a standard practice in sugarcane cultivation (Lee, Pierson and Kennedy 2002).

Inoculation of rice seedlings with *Burkholderia Vietnamiensis* increased grain yield in field studies (Tran Van, Berge, Ke, Balandreau and Huelin 2000), and this bacterium is capable of contributing 25–30 kg N/ha. Under gnotobiotic conditions this species can fix 19% of the nitrogen required by the rice plant, while another *Burkholderia* sp. was reported to fix 31% of the nitrogen the rice plant required and increase plant biomass by 69% (Baldani, Baldani and Dobereiner 2000).

17.2.4 Interaction with Mycorrhiza

Vesicular-arbuscular mycorrhizal (VAM) fungi are characterized by limited growth within the roots and extensive growth of the hyphae beyond the root zone. VAM fungi can improve plant vigor, nutrient and water uptake, disease resistance and drought tolerance. The principal contribution of the fungi is assistance in phosphorous acquisition, particularly in phosphorous-depleted soil, and other trace elements (Boddington and Dodd 1998, 1999; Clark 1997). Depending upon soil phosphorous content and crop plant the VAM inoculant application can reduce 25–50% P-fertilization cost (Tiwari, Adholeya and Prakash 2004). Some rhizobacteria have been identified that promote VAM development by enhancing receptivity of the root to VAM fungi and triggering germination of the VAM fungal propagules (Garbaye 1994). VAM improved nodulation of several legumes (Barea, Escudero and Azcon-G de Aguilar 1980; Smith and Bowen 1979), and enhanced N_2-fixation by rhizobia (Chaturvedi and Kumar 1991; Werner, Berbard, Gorge, Jacobi, Kape, Kosch, Muller, Parniske, Scenk, Schmidt and Streit 1994), *Azotobacter* (Alnahidh and Gomah 1991), and *Frankia* (Sempavalan, Wheeler and Hooker 1995). Further information about the synergy between VAM and beneficial rhizobacteria and their potential for stimulating plant growth is given in a recent review of Arturrson, Finlay and Jansson (2006).

17.3 Biofertilizers in Crop Production

Positive effects of PGPR, typically referred to as biofertilizers, seed inoculation have been reported in a variety of crops (Table 2) and have been shown to reduce plant stress (Table 3).

The use of biofertilizers in rice production has been extensively studied. Diazotrophic rhizobacteria that are commonly associated with rice include *Azospirillum, Herbaspirillum* and *Burkholderia* (Baldani et al. 2000; Balandreau 2002; Malik, Mirza, Hassan, Mehnaz, Rasul, Haurat, Bslly and Normand 2002). These diazotrophs, including cyanobacteria, can substantially contribute to the nitrogen requirements of rice plants. Watanabe, Yoneama, Padre and Ladha (1987), and Roger and Ladha (1992) concluded that BNF can provide up to 25% of the nitrogen requirement of rice.

In Vietnam a biofertilizer consisting of *Ps. fluorescens*/*Ps. putida* (BNF), *Klebsiella pneumoniae* (anaerobic BNF, PO_4-solubilizer) and *Citrobacter freundii* (BNF) is used in rice production. *Citrobacter freundii* is also antagonistic to 50% of the common rice rhizospheric bacteria, but not to the other components of biofertilizer, which aids in the establishment of the inoculum (Nguyen, Kennedy and Roughley 2002). This biofertilizer significantly increased grain yield (21% over control) and nitrogen accumulation (Nguyen, Deaker, Kennedy and Roughley 2003). In another field study, a biofertilizer containing two cyanobacteria (*Anabaena* and *Nostoc*), *Azospirillum* sp. and *Azotobacter* sp. applied with a third of the recommended amount of urea fertilizer produced greater rice grain yield than any single component of biofertilizer and/or nitrogen fertilizer (Yanni and Abd El-Fattah 1999). Other multi-strains biofertilizers were used in Pakistan (Malik et al. 2002) and Egypt (Hegazi et al. 1998). Overall, the reported increased rice grain yield due to biofertilizers was about 20%.

Table 2. Selected examples of growth-promoting rhizobacteria (PGPR) on plant growth and production

Plant	PGPR	Response	Reference
Bean	*Pseudomonas putida*	Increased overall performance	Anderson and Guerra 1985
Canola	Unknown	Increased overall performance	Kloepper 1994
Canola	*Pseudomonas putida*	Increased root and shoot length; increased dry weight, chlorophyll and protein content	Glick et al. 1997
Corn	*Pseudomonas aeruginosa,* *P. fluorescens*	Enhanced seed germination and dry matter accumulation	Hofte et al. 1991
Cotton	*Pseudomonas cepacia*	In the field suppressed *Rhizoctronia soloni*; equivalent to a fungicide treatment; and significantly increased seedling stand	Press and Kloepper 1994
Cotton	*P. fluorescens*	Nematicide against *Rotylenchulus reniformis* and increased growth	Jayakumar et al. 2003
Peanut	*Bacillus subtilis*	Increased yield	Turner and Backmann 1991
Potato	*Pseudomonas* sp.	Increased yield	Geels and Schippers 1983
Rice	*Pseudomonas fluorescens*	Decreased sheath rot	Sakthivel et al. 1986
Rice	*Pseudomonas fluorescens*	Decreased bacterial blight	Velusamy et al. 2003
Spring wheat	*Bacillus* sp.	Increased shoot height and root growth under controlled conditions	Chanway et al. 1988
Spring wheat	*Bacillus* sp.	Increased tiller number and yield	Grayston and Germida 1994

Table 3. Examples of plant growth promoting rhizobacteria (PGPR) reducing plant stress

Plant	Stress	PGPR	Reference
Barley	Heavy metal	*Arthobacter mysorens* *Flavobacterium* sp. *Klebsiella mobilis*	Pishchik et al. 2002
Soybean	Cool soil temperature	*Serratia proteamaculans* *Serratia liquefaciens* *Aeromonas hydrophila*	Zhang et al. 1997
Loblolly pine	Ozone	*Bacillus subtillis* *Paenibacillus macerans*	Estes et al. 2004
Tomato	Salt	*Achromobacter piechaudii*	Mayak et al. 2004
Wheat	Salt	*Azosprillium lipoferum*	Bacilio et al. 2004
Arabidopsis	Water	*Paenibacillus polymyxa*	Timmusk and Wagner 1999

Corn production requires significant amounts of nitrogen. Diazotrophs commonly found in the corn rhizosphere include *Enterobacter, Rahnella aquatilis, Paenibacillus, Azotofixans, Azospirillum, Herbaspirillum seropediacae, Bacillus circulans and Klebsiella* (Chelius and Triplett 2000), and these diazotrophs can contribute significant amounts of nitrogen (Garcia de Salamone, Dobereiner, Urquiaga and Boddy 1996). Application of biofertilizer containing *A. brasilense* increased corn yield 50–95% (0.7–1.0 t/ha) depending on soil nitrogen status. Corn seed inoculation with *H. seropediacae* increased grain yield in greenhouse experiments by 49–82% when nitrogen was added, while only a 16% increase was observed without fertilizer. This indicated that the inoculum improved nitrogen assimilation by the plant (Riggs, Chelius, Iniguez, Kaeppler and Triplett 2001). Application of the inoculant in field experiments at different U.S. locations increased corn yield up to 20% (Riggs et al. 2001). Seed inoculation with a selected strain of *Burkholderia cepacia* enhanced corn yield 6% in field experiments; yield increase in greenhouse test using non-sterile soil varied between 36 and 48% depending on host cultivar and bacterial genotype (Riggs et al. 2001).

Sugarcane, like corn, is a nitrogen-demanding crop. Diazotrophs commonly associated with sugarcane include: *Acetobacter diazotrophicus, Azospirillum brasilense, A. linoleum, A. amazonense, Bacillus brasilensis, Burkholderia tropicalis, Herbaspirillum seropediacae* and *H. rubrisubalbicans* (Ries, Ries, Urquiaga and Dobereiner 2000; Sevilla and Kennedy 2000; Kennedy and Islam 2001). Application of diazotrophic PGPR (in soil or as a settes inoculation) can significantly reduce the amount of fertilizer nitrogen required for sugarcane production (Dobereiner 1997). Boddey, Polidoro, Resende, Alves and Urquiaga (2001), using ^{15}N

natural abundance technique, showed that BNF can contribute 60% of nitrogen assimilated by sugarcane not receiving fertilizer nitrogen. Dobereiner (1997) concluded that BNF can contribute up to 150 kg N/ha. Inoculation of sugarcane settes with biofertilizer (containing diazotrophs *Acetobacter diazotrophicus, Herbaspirillum* sp., *Azospirillum lipoferum* and a vesicular arbuscular mycorrhiza) in field experiments, which received 50% of the recommended nitrogen fertilizer, produced cane yields that were not significantly different from those that received the recommended amount of the fertilizer. It was suggested that the diazotrophs may have contributed the majority of plant's nitrogen requirement, as well as produced appreciable amounts of IAA that promoted rooting and improved growth, and that using biofertilizer could reduce the application of nitrogen fertilizer by 50% without yield loss (Muthukumarasamy, Revathi and Lakshminarasimhan 1999). The examples provided thus far have illustrated the use of PGPRs in crop production; however, there has also been extensive use of PGPRs and mycorrhizal fungi in forestry applications.

Examples of PGPRs used in forestry are provided in Table 4. Several PGPRs have been used to improve container growth and reduce transplant shock. Black oak seedlings inoculated with *Pisolithus tinctorius* improved seedling survival, growth in reforestation sites, and drought tolerance compared to bare root stock (Dixon, Wright, Garrett, Cox, Johnson and Sander 1981, 1983). Even at low colonization levels, American ash inoculated with *Glomus epigaeum* increased the seedling growth and dry weight (Furlan, Fortin and Planchett 1983). Pine seedling inoculated with *Pisolithus tinctorius,* and sawforth oak with *Thelephora terrestris*, enhanced seedling survival and increased plant height and diameter compared with natural inoculation in the field (Anderson, Clark and Marx 1983). Leucaena inoculated with *G. etunicatum* promoted its establishment under low fertility level (Tomar, Shrivastava, Gontia, Khare and Shrivastava 1985), and Thapar and Khan (1985) reported a significant increase in growth and dry weight of hoop pine seedlings grown in soil inoculated with VAM fungi.

As indicated earlier, there is a synergism between VAM and PGPRs. Inoculation of oak seedlings with *Azotobacter* was reported to be beneficial (Panday, Bahl and Rao 1986). Dual inoculation of leguminous trees with rhizobia and VAM fungus improves growth of the trees compared with plants inoculated with either inoculant alone. Significant growth increase of velvet wattle (66%) and of acacia (16%) resulted from seedling inoculation with *Rhizobium* sp. and *Glomus mosseae*, compared with rhizobia inoculation alone (Cornet and Diem 1982). The role of mycorrhiza in trees and the roles their symbioses play in forestry have recently been reviewed (Dahm 2006).

Table 4. Examples of growth-promoting rhizobacteria (PGPR) in forestry

Plant	PGPR	Response	Reference
White spruce, Lodge pole pine	*Bacillus* sp.	Increased seedling emergence, shoot height and weight, root surface area and weight.	Chanway et al. 1991
Lodge pole pine	*Bacillus* sp. and *Wilcoxina miklae* (mycorrhiza) (co-inoculation)	Increased shoot biomass and foliar nitrogen content.	Chanway et al. 1991
Pine, Spruce	Unidentified bacteria	Promoted growth; increased seedling biomass.	Chanway 1997
Loblolly pine	Unidentified bacteria	Reduced fusiform rust infection.	Enebak and Carey 2004
Jeffrey pine	*Pisolithus tinctorius*	Promoted root and shoot growth; increased nutrient uptake.	Walker and Kane 1997
Loblolly pine, Slash pine	Unidentified bacteria	Increased biomass. Promoted root and shoot growth.	Enebak et al. 1998
Loblolly pine	*Bacillus subtilis* *Paenibacillus macerans*	Protected against negative effects of ozone exposure.	Estes et al. 2004

17.4 Inoculum Preparation and Application

The potential of biofertilizers to increase plant growth and yield in controlled environments and the field is well documented. However, examples of inconsistent results are also reported. Inadequate colonization of the host rhizosphere by the introduced agents is probably the principal reason for inconsistencies in the expected results from field application of biofertilizers. Availability of soil nutrients, phosphate in particular, soil pH and moisture content are important factors influencing the survival, proliferation, and host-plant root occupancy. West, Burges, Dixon and Wyborn (1985) reported that soil nutrient availability was the most important factor in the survival of *Bacillus thuringiensis* and *B. cereus.* A better understanding of microbial ecology of the host rhizosphere in the presence of the introduced inoculant is essential before biofertilizers can become regular agriculture practice (Lazarovits and Nowak 1997).

Peat moss has been a popular carrier material for inoculant bacteria, but any suitable locally available material may be used. For example, finely pulverized ricehusks are used in several Asian countries. The addition of bentonite clay to the carrier material promoted bacterial survival in fine textured soil (England, Lee and Trevors 1993). Chemical polymers for entrapping inoculant bacteria and application for subsequent colonization of the rhizosphere have shown promising results. Addition of other soil amendments may also encourage colonization. In one case, barley straw used as a soil additive promoted survival of the inoculant bacteria and improved root colonization (Stephens 1994).

The physiological status of the bacteria prior to application (mixing with the carrier material) appears to influence the survival and colonization. Application of the bacterium from the late exponential growth phase resulted in higher stabilization and reduced mortality compared to bacteria taken from an earlier growth phase (Vandenhove, Merchx, Wilmots and Vlassak 1991). Heijnen, Hok-A-Hin and van Veen (1992) found that mixing freeze-dried or fresh-grown *R. leguminosarum* cells with 1% bentonite clay prior to introduction to the soil markedly enhanced bacterial survival compared to treatments without the amendment. Starved cells introduced into sandy loam soil significantly enhanced *P. fluorescens* survival and wheat root colonization as compared to fresh cells (Heijnen, Hok-A-Hin and van Elsas 1993). Further research in the area is warranted.

Very few references concerning the delivery of the inoculant and the establishment of an effective population are available. It is known that the population density of the inoculum in the rhizosphere is often proportional to the initial load of inoculum on seed (Milus and Rothrock 1993). Although increasing the amount of inoculum used does increase the potential for a greater population in the rhizosphere, the results are not always consistent (Hebber, Davy, Merrin, McLoughlin and Dart 1992). Introduced bacteria must colonize their new soil-root environment while competing with indigenous microbes. For this reason, competitive ability and greater growth rate of the introduced inoculum in the rhizosphere are considered desirable traits in selecting a strain of inoculant bacteria. The root colonization is a competitive process affected not only by the characteristics of the introduced inoculant and the host, but also soil abiotic and biotic factors in the rhizosphere and their interactions. Few studies have been attempted to develop a screening method for identification of strains of selected bacteria (associative diazotrophs, PGPR, phosphate solubilizer, etc.) capable of establishing and maintaining an effective population density in the host rhizosphere throughout the life cycle of the host (Nijhuis, Maat, Zeegers, Waalwijk and Van Veen 1993). Commercial rhizobial inoculants usually contain multiple strains. Use of multiple strains of an inoculant bacterial species may enhance host plant root colonization; however it can not be recommended prior to field verification.

17.5 Commercial Availability of Biofertilizers

Tiwari et al. (2004) published a list of 35 sources of commercial biofertilizer. Twenty-four of these companies were located in North America. Of the remainder, six were located in Europe, two each in Asia and India, and one in South America. A fairly extensive internet search in 2006 revealed that 16 of these 35 companies were still actively producing and marketing biofertilizer. Seven of the other 19 had ceased production and sales of biofertilizer, but continued marketing other products. The other 12 companies were either no longer in business or had merged with other corporations.

Results of our 2006 search did, however, consist of a total of 49 sources of biofertilizer in the following locations: 38 in North America, five in Europe, three in India, two in Asia, and one in South America. There may be other sources available that lack an internet site. A representative sample of commercial suppliers is provided in Table 5. Table 6 lists some of the most common uses of biofertilizer, while Table 7 lists some of the typical organisms used. The majority of the products are used for stimulation of growth (23%), insect control (21%), or disease management (14%). Although there appears to be a variety of commercial biofertilizers available, the internet and literature searches did not find many references as to their use in practical applications or recommendations for their use as part of a management practice.

Table 5. Selected biofertilizer companies

Company name	Location	Web address
ABTEC[1]	India	www.abtecbiofert.com
Accelerator Horticulture	USA	www.webberlandscape.com
Advanced Green	Taiwan	itrademarket.com
Aureus Biotech	Singapore	www.aureustech.com
Biocontrol Network	USA	www.biconet.com
BioFertilizer, Inc	Costa Rica	www.biofertilizer.com
BioMax	India	www.indiamart.com
BioOrganics	USA	www.bio-organics.com
BioRize	France	www.biorize.com
Cleary Chemical	USA	www.clearychemical.com
EM America	USA	www.emamerica.com
EuroAgro	Holland	www.euroagroec.com
Horticultural Alliance	USA	www.hortsorb.com
J.H. Biotech	USA	www.jhbiotech.com
Nafed BioFertilizer	India	www.nafed-india.com
Natural Industries	USA	www.naturalindustries.com
PlantWorks, Inc	UK	www.plantworksuk.co.uk
Premier Horticulture	Canada	www.premierhort.com
Prophyta GmbH	Germany	www.prophyta.de
Rhode's Nursery	USA	www.beorganic.com
Rizobacter Argentina S.A.	Argentina	www.rizobacter.com.ar
Roots, Inc	USA	www.rootsinc.com
Sri BioTech	India	www.sribio.com
Verdera	Finland	www.verdera.fi/homeeng.html

[1] Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Table 6. Typical uses of biofertilizers

Use	Percentage of products
Disease Control/Resistance/Suppression	14
Establishment/Vigor	13
Fungicide	8
Growth Stimulation	23
Insecticide	21
Nematicide	3
Nitrogen Fixation	5
Nutrient Uptake/Availability	6
Phosphorous Solubility	1
Stress Resistance	3
Yield	3

Table 7. Organisms used in biofertilizers and their typical use

Organism	Use
Acetobacter sp.	Nitrogen Fixation
Aspergillus sp.	Nutrient Uptake/Availability
Athrobacter sp.	Growth, Vigor
Azospirillum sp.	Yield
Azotobacter sp.	Establishment/Vigor
Bacillus sp.	Growth, Insecticide, Fungicide
Beauvaria sp.	Insecticide
Gigaspora sp.	Growth
Gliocladium sp.	Fungicide
Glomus sp.	Growth
Paecilomyces sp.	Nematicide
Phosphobacteria sp.	Phosphorus Solubilization
Pisolithus sp.	Growth
Pseudomonas sp.	Disease Control
Rhizopogon sp.	Disease Suppression
Trichoderma sp.	Fungicide

While biofertilizers are clearly potentially useful, it is apparent that a gap exists between research done by scientists and application in agricultural practices. Part of this may be due to the inconsistencies of the results between laboratory and field studies. It may be that we lack sufficient field studies to determine the beneficial effect of biofertilizers, or that our understanding of rhizosphere dynamics is too limited to understand the conditions required to establish a PGPR. It might be helpful if we evaluated commercially available biofertilizers in the field to establish the range of soils, environments, and management practices that limit their practical application. However, we may find that PGPRs are more useful during stress conditions or marginal production conditions. For example the use of PGPRs to enhance soybean seedling growth and nodulation under cool-soil temperature conditions (see Table 3). The use of biofertilizers in marginal or stress conditions needs to be evaluated further. Their use in resource-limited applications (reduced fertility, minimum input systems) may be of greater benefit than when biofertilizers are used in conjunction with best management practices.

The gap between discovery of PGPRs, development of biofertilizers and their application may also be the result of inadequate technology transfer and limited farmer education. The Forum for Nuclear Cooperation in Asia held a technical meeting in June 2005 to evaluate the status of biofertilizer use in several Asian countries. In the meeting's summary, Thailand reported a problem with public relations and technology transfer as limiting biofertilizer use, while Indonesia cited lack of education for farmers as a primary problem. However, the countries outline plans to increase biofertilizer education for farmers and public relation efforts to try to make biofertilizer a more attractive option to the local agriculture community. Adequate efforts must be made to translate this research into forms easily adapted to and adopted by farmers in order for biofertilizer to be a viable long-term aspect of the agriculture industry.

17.6 Conclusions

The potential of PGPRs for enhancement of plant growth and yield, and their role in weed and disease suppression is well documented. However, inconsistencies in the effectiveness of PGPR inoculants between laboratory and field studies are a major impediment to their application in agricultural practices (Schroth and Becker 1990; Burdman, Vedder, German, Itzigsohn, Kigel, Jurkevitch and Okon 1998). The complexities of the plant-soil interactions and the dynamics of the rhizosphere organisms need to be more fully understood before the potential of PGPRs can be exploited. Further field studies with known PGPRs and commercial biofertilizers are needed to determine their effectiveness. Encapsulation, product shelf-life, and application methods need further evaluation. Finally, management practices incorporating PGPRs need to be designed and demonstrated as useful in crop production. When nitrogen fixing bacteria were introduced in legume production it took over 30 years to develop the technology to its present level. Effective strains, host compatibility, commercial preparation, and the transfer of the technology require time. We can use this experience to develop biofertilizers and established their use in achieving a sustainable agriculture.

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18. Utilization of Stress Tolerant, Weed Suppressive Groundcovers for Low Maintenance Landscape Settings

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Abstract. The recent emphasis on development of alternative, non-chemical weed strategies for landscape and roadside management has led to the study and utilization of well-adapted landscape groundcovers, including turfgrass and herbaceous ornamentals that can successfully withstand and suppress weed invasion. By selecting groundcovers which exhibit growth characteristics that result in consistent interference with weed establishment, one can successfully achieve effective long-term weed control in the landscape using reduced herbicide and labor inputs. In addition to allelopathic characteristics resulting in enhanced weed suppression due to the production and release of phytoinhibitors in the landscape, some groundcovers effectively suppress weedy invaders through competition for space, sunlight, moisture, nutrients and even direct alteration of the rhizosphere environment. Additional information related to weed biology and the impact of cultural practices used in the landscape on weed infestation are also critical when considering long-term and sustainable weed suppression for landscape and natural settings. Until recently, little information was available regarding the development of low maintenance landscapes for use in the Northeastern United States, with the exception of roadside trials documenting utilization of certain direct-seeded groundcovers in highway medians. Recent studies in both landscape and roadside settings have indicated that the selection and utilization of certain herbaceous perennial groundcovers and turfgrasses may not only result in lower labor inputs with respect to maintenance, they may further prevent the spread of noxious invasive weeds by severely limiting their ability to establish. When surveyed, stakeholders reported that weed suppressive groundcovers had much greater aesthetic appeal than those that were partially infested. Many of these groundcovers suppress weeds effectively by intense competition for resources, particularly by reduction in the amount of available light at the soil surface due to dense canopy cover. In addition, some groundcovers such as *Nepeta x faasennii* and *Festuca rubra* inhibit weed seedlings by their potential release of allelochemicals into the rhizosphere. Although still relatively novel, allelopathic groundcovers and turfgrasses offer interesting possibilities for future development of allelochemicals as bioherbicides and attractive, stress-tolerant and pest-resistant landscape plantings.

18.1 Landscape Weed Ecology

Weeds consistently appear in less well-maintained landscape, roadside and turf settings, particularly in stressed sites or unattended landscape beds. To manage vegetation with reduced pesticide, fertilizer and labor inputs, we recommend a better understanding of the reasons why weeds often persist in landscape settings (Bertin and Weston 2004, 2006). Key factors involved in successful weed establishment in the landscape include: (1) a weed's ability to germinate or propagate vegetatively, (2) availability of resources and space, (3) repeated disturbance of the soil or seedbed, (4) presence of neighboring vegetation, (5) presence of other pests and herbivores and (6) current environmental conditions. A greater understanding of the landscape ornamental or groundcover's interactions with the environment, including the rhizosphere environment and the impact of stress on plant growth is critical when developing effective strategies to manage weeds with reduced inputs (Mortensen et al. 2000). In low maintenance landscapes and roadside settings, the most critical time for effective weed management is before establishing the landscape or seeding the turf or groundcover. Effective planning and design involves the selection of appropriate plant materials that are well- suited to the location of establishment (Bertin and Weston 2004). Effective establishment is dependent upon plant and seed quality, timing of establishment, environmental conditions at the time of establishment and preparation of the site, including removal of existing weeds (Bertin and Weston 2004; Eom et al. 2005). By selecting plant materials that are well-adapted to the landscape site, future weed infestation may be greatly reduced, as ornamentals successfully outcompete encroaching weeds for space and resources.

Although herbicides are still a predominant means for managing weeds in many commercial and private landscapes and roadside settings, greater emphasis has now been placed on the utilization of alternative or non-chemical means for weed management, for many reasons (Bertin and Weston 2004; Matteson 1995). With respect to the public sector, the largest landscape site to be maintained is along managed highway and road networks. For example, the cost of vegetation management along New York State's roads and highways results in expenditures of millions of dollars per years for herbicides, mowers, labor, and hand-pulling or trimming vegetation (Eom 2004; Ropel 2004). With declining resources, the management of noxious or invasive weedy species is very challenging, and often requires large inputs in labor for mowing, chopping and removal. In some instances, hand removal of noxious weeds can be dangerous to landscape managers. For example, giant hogweed (*Heracleum mantegazzianum*), an invasive weed now infesting areas across the Northeastern United States and Canada, has been proven to be dangerous due to its ability to cause severe dermatitis upon contact (New York State Department of Agriculture and Markets publication 2005). Specifically, the plant releases exudates containing furanocoumarins from its cut foliage, which can result in serious skin injuries or burns when photosensitization or exposure to light occurs in the presence of the compounds. In some cases, severe burning has resulted in tissue necrosis and extensive damage to exposed skin. Although hand removal and mowing of weeds is not always practical or feasible, concern about herbicide safety and persistence has led some municipalities in the United States and Canada to ban the use of herbicides in all public areas, despite increased weed pressures in landscape and roadside settings. However, with the increasing spread of invasive and noxious species across the North American landscape, greater attention to the development of effective management alternatives is clearly needed (Westbrooks 1998).

By using groundcovers, which exhibit consistent weed suppressive growth characteristics, one can attempt to achieve effective long-term weed management in the landscape using reduced herbicide and labor inputs (Eom 2004). In addition to allelopathic characteristics resulting in enhanced weed suppression due to the production and release of phytoinhibitors in the landscape, certain groundcovers suppress weedy invaders through effective competition for space, sunlight, moisture, nutrients and even direct alteration of rhizosphere ecology (Weston and Duke 2003). Additional information related to weed biology and the impact of such cultural practices used in the landscape as trimming, mowing, fertilization, irrigation and mulching are important to evaluate methods for long-term and sustainable weed suppression in landscape and natural settings (Bertin and Weston 2004). Until recently, not much research has been devoted to the development of low maintenance, low input landscapes for use in the Northeastern United States, with the exception of roadside trials documenting utilization of groundcovers along highway medians (Ropel et al. 2004). However, our recent work in both landscape and roadside settings has indicated that the selection and utilization of newly released plant materials to provide effective suppression of weeds over time may not only result in lower labor inputs with respect to maintenance, they may further prevent the spread of noxious invasive weeds by severely limiting their ability to establish in landscape settings (Eom et al. 2005). However, it is important to consider the ramifications if one utilizes a very competitive groundcover or landscape planting that has the potential to be inherently invasive as well. Therefore, careful selection and utilization of available plant materials and groundcovers is critical when establishing a sustainable landscape or roadside setting (http://www.entomology.cornell.edu/Extension/Woodys/CUGroundCoverSite/GroundcoverMain.html).

This chapter will focus upon the recent information available with respect to the selection of both native and non-native weed suppressive turfgrasses and herbaceous groundcovers for use in the landscape, natural areas or roadside settings. One of first research objectives when we began our studies with little utilized species as groundcovers was to evaluate the success of both transplanted and direct-seeded materials in settings receiving minimal landscape maintenance. These settings can be very diverse and have included both full-sun and shaded sites, as well as well-traveled roadside locations. Recent research performed by our laboratory group and others has shown that certain groundcovers that are well-adapted to the location can establish densely, suppress weeds and provide an attractive, easily maintained stand over a multi-year time frame (Eom et al. 2005). Many are also very resistant to infestation by other pests, including herbivores (Weston 1999). We will also discuss the successful characteristics associated with highly weed suppressive groundcovers requiring limited on-site maintenance and describe potential sites where their successful establishment offers additional opportunities for their utilization in landscape and naturalized areas.

18.2 Weed Suppressive Herbaceous Perennial Groundcovers

Perennial herbaceous groundcovers are generally established in landscape plantings using transplants produced from seed or by division. Many newly available groundcovers are currently being studied with great interest as alternative plant material selections for gardens, roadsides, reclamation areas and xeroscapes (Weston 1999; Weston et al. 2006). Groundcovers are utilized for many purposes including their ability to shade the soil, prevent soil erosion, suppress weed growth, and improve the aesthetic appeal of diverse landscapes (Eom et al. 2005; Weston et al. 2006). Herbaceous perennial groundcovers can be relatively easy to establish and maintain, and those that tolerate environmental extremes are of increasing interest to gardeners and landscape maintenance personnel (Armitage 1997; MacKenzie 1997). In the Northeast and Central United States, the ability to suppress weeds and resist insect, disease, and deer browsing pressures are also important criteria for successful establishment and utilization in low maintenance landscape settings. In comparison to the establishment of the most commonly utilized groundcover––a mixed turfgrass sod–herbaceous perennials can provide reduced maintenance opportunities and greater aesthetic appeal (Weston et al. 2006).

Given recent extreme environmental conditions encountered across the United States involving periods of extended drought and excessive rainfall events, as well as temperature extremes, plant materials that tolerate these stressful conditions are gaining popularity as landscape plantings (Usón and Poch 2000). It has been suggested that recent global warming trends may exacerbate stressful conditions in many locations across the globe. Recent studies have shown that some herbaceous groundcovers can successfully tolerate such stressful conditions. Herbaceous groundcovers can be transplanted in such challenging sites as those encountered along roadsides or driveways, on steep slopes, in dense shade or full sun, or even along bodies of water (Armitage 1989; MacKenzie 1997; Weston 2005). Recently, we have evaluated an extensive collection of herbaceous perennial groundcovers for use in low- maintenance settings, under full sun conditions in New York. We monitored their ability to establish successfully and over winter; produce adequate biomass or coverage of the soil surface; and resist disease, insect infestation, and deer browsing. In separate greenhouse experiments in New York, we also evaluated the ability of selected groundcovers to tolerate drought and salt stress (Eom et al. 2007). As weed scientists and ecologists, we were particularly interested in interactions between weeds and groundcovers, as well as the ability of these relatively uninvestigated plant materials to effectively suppress weeds over time, especially in challenging landscape environments (Eom et al. 2005).

After several years of field and roadside experiments supported by the New York State Department of Transportation and the Horticulture Research Institute, we determined that groundcovers that consistently possessed certain growth characteristics were most effective in suppressing weeds over time. Not surprisingly, those groundcovers that rapidly established by producing a dense foliar canopy, which effectively prevented light from reaching the soil surface, were the most effective in providing long-term weed suppression. In addition, groundcovers that maintained consistent growth after establishment and formed a closed canopy while achieving a height of 10 inches or more were generally infested with fewer weeds and less weed biomass (Eom 2004; Weston et al. 2005).

Weeds typically establish in areas where adequate space and moisture are available, and weed seeds are often stimulated to germinate when light intercepts the soil surface. Creeping phlox (*Phlox subulata*) “Emerald blue”, lady’s mantle (*Alchemilla mollis*), catmint (*Nepeta* spp.), lamb’s ear (*Stachys byzantina*), and coral bells (*Heuchera americana*) “Chocolate Veil” rapidly and consistently formed very dense foliar canopies that prevented light from reaching the soil surface, and were not easily infested with weeds, either when maintained with no maintenance or some initial hand weeding. Groundcovers that were slightly taller (at least 10 inches tall) were generally not as easily dominated by escaped weeds, which can tower over creeping or prostrate groundcovers, despite their dense establishment. Prostrate groundcovers such as creeping speedwell (*Veronica repens*), various baby's breath (*Gypsophila* spp.), and purple clover (*Trifolium* spp.) tended to be more easily weed-infested, especially in year one of our studies. Other groundcovers, including those presented in Table 1, were generally well established and suppressed weeds over time when initial weeding was provided to assist establishment. These included creeping thyme (*Thymus praecox*), *Dianthus* spp., dwarf goldenrod (*Solidago sphacelata*), sedum (*Sedum reflexum*), and fragrant sumac (*Rhus aromatica*). In the case of *solidago sphacelata* and *sedum reflexum*, growth pattern has shown rather poor establishment in some case in subsequent years, although they were well established in the first year. However, related cultivars such as ornamental goldenrod (*Solidago cutleri*) and sedum (*Sedum acre*) were well established over several years. Certain species also exhibited greater tolerance to stressful conditions when evaluated in controlled greenhouse conditions for drought and salt tolerance. In addition to being drought tolerant, ornamental goldenrod was exceptionally tolerant to high salt concentrations, which might routinely be encountered along roadside settings (Eom et al. 2007). Groundcovers that were succulent or possessed leaf types that limited evapotranspiration were generally most drought tolerant; these included the succulent sedum, lamb's ear, creeping thyme, creeping phlox, and ornamental goldenrod (Eom 2004; Weston et al. 2006).

Recently, several herbaceous groundcovers have been noted in the literature for their ability to potentially suppress weeds by the allelochemicals they release (Weston 2005). Extracts of creeping phlox foliage, ornamental goldenrod, and catmint have been shown to contain plant growth inhibitors that effectively suppress weed seedling growth (Shiraishi et al. 2002; Eom et al. 2006). In the case of catmint, as well as other members of the mint family, large quantities of volatile chemicals are released from glands on the leaf surface. The volatile or gaseous mixture, likely released by the plant upon disturbance or rainfall events, contains several related nepetalactones, terpenoids, sesquiterpenoids, and phenolic compounds. The mixture is very active in suppressing weed seedling growth and is also reported to reduce insect herbivory. These volatiles can be easily detected just by walking through or brushing a planting of catmint, which gives off a distinct odor from its foliage (Eom et al. 2006). Herbaceous ornamentals such as purple and orange coneflower (*Rudbeckia* spp.) and other members of the sunflower (*Helianthus* spp.) family have also been noted to be weed suppressive due to allelochemicals released from their foliage, flowers, and stems (Weston 1999, 2005).

The secondary compounds produced by herbaceous perennials can also contribute to their ability to resist deer browsing and insect infestation. In our roadside trials, we observed that Missouri primrose (*Oenothera missouriensis*) and other *Onoethera* spp. were attractive to browsing deer, whereas catmint, lady's mantle, and creeping phlox were never disturbed (Weston 2005). In insect surveys performed in established groundcovers, we observed that many species remained free of insect damage over time. Other species produced colorful inflorescences that attracted a variety of bees, butterflies and other beneficial insects throughout the growing season. Very few species, if any, were adversely affected by the presence of damaging insects. This might be explained by the fact that many groundcovers evaluated had thick pubescence or waxy cuticles on their leaf surfaces that deter insect herbivory, in addition to the secondary products they may produce in their foliage (Eom et al. 2006).

Recently Cardina and Krohn evaluated cover crops for use in vineyards and other horticultural sites in Ohio. They found that grasses and legumes developed for pastures were often very competitive with the crop, but low-growing species like creeping mazus (*Mazus reptans*) provided good soil coverage and weed suppression without ill effects on the crop. Since then over 100 species for agricultural and home landscape applications have been evaluated in Ohio (Weston et al. 2006).

In the Ohio studies, groundcovers were limited in height to about 4 inches, since the goal was to create low-maintenance groundcovers as alternatives to grasses. Although results are preliminary, species that look promising in this somewhat warmer climate, include creeping mazus, blue star creeper (*Laurentia fluviatilis*), and cinquefoil (*Potentilla tridentata*) "Nana", all of which are low-growing, creeping, herbaceous species. A few semi-woody species like heath aster (*Asteracea ericoides*) and the less common fleeceflower or pink poker (*Persicaria affinis*) also look interesting in terms of their ability to rapidly cover the soil surface and form a dense canopy. Part of the focus of the Ohio work is to evaluate species mixtures and to determine if there are some "rules" of plant growth habit and life history that make the most successful mixtures. Sometimes big patches of a single species tend to die out in the center, especially for plants like dianthus, some veronicas, and lysimachia (*Lysimachia nummularia*). However, when species are allowed to establish together in mixed culture, the plant growth habit can change, and this might make them less susceptible to brown-out (Weston et al. 2006).

Two problems that landscape managers consistently face with low-growing groundcovers, including turfgrass, are establishment and weed management. Many of the species we have tested must be transplanted and initial establishment can be slow. This means that spaced plantings take some time to fill in, during which time weeds can get a foothold (Weston 2005). Broadleaf weed control in turfgrass is fairly easy with selective postemergent herbicides, but in a broadleaf groundcover managers often rely on hand weeding as herbicides are non-selective. Since groundcovers are less competitive and die back during the fall, annual winter weeds can become established if the canopy is not as dense. Consideration must therefore be given to spacing or higher planting density to achieve best results in terms of weed suppression (Eom et al. 2005).

Greater consideration of new plant materials including groundcovers for retail or wholesale production by the nursery industry may also be warranted. In a recent consumer survey performed on Long Island with participants who viewed weeded and unweeded perennial groundcovers, our results showed that consumers were not only highly concerned about herbicide usage in the landscape, but were greatly interested in low-maintenance plant materials for establishment. In addition, groundcovers that were inherently more weed suppressive received significantly higher rankings for their appearance and overall aesthetic appeal, and were of greater interest to consumers from the standpoint of desirability for use in their own landscapes (Allaire 2005; Weston et al. 2006).

Our favorite herbaceous perennial groundcover selections, based on their consistent aesthetic appeal and weed suppression, as well as their relative ease of establishment, are presented in Table 1. These groundcovers have been evaluated in numerous trials and demonstrations across New York and/or Ohio. The list presented is certainly not inclusive, as ongoing research will no doubt reveal other new, useful plant materials for low maintenance establishment. However, with increasing emphasis on reduction of pesticide use in both private and public landscape settings, and time and labor constraints in landscape maintenance, the use of new perennial plant materials in diverse landscape and horticultural settings will undoubtedly be of greater importance in future years. While it is unlikely that groundcovers will replace the vast expanse of mowed turfgrass in the landscape, they could be utilized effectively in many open areas that don't require the durability of turf species (Fig. 1). For more information on these and other groundcovers, please visit our website: http://www.entomology.cornell.edu/Extension/Woodys/CUGroundCoverSite/GroundcoverMain.html (Weston 2006).

Table 1. Selected groundcovers that were consistently well-established, weed suppressive, and aesthetically appealing in landscape and roadside trials

Common name	Scientific name	Growth habit	Description
Albanian pinks	*Dianthus myrtinervius*	4" tall 10" spread	Attractive, low-growing, pink flowers
Blue star creeper	*Laurentia fluviatilis*	2" tall	Attractive blue flowers all summer
Catmint "Walker's low"	*Nepeta x faassenii*	12–20" tall 16" spread	Tall, rapid growth rate; attractive blue flowers
Cinquefoil "Nana"	*Potentilla neumanniana*	3–4" tall	Five shiny leaflets, yellow blooms
Coral bells "Chocolate Veil"	*Heuchera americana*	18" tall	Dense canopy of chocolate – colored foliage
Creeping phlox "Emerald blue"	*Phlox subulata*	<6" tall	Attractive green foliage and flowers
Creeping thyme	*Thymus serpyllum*	<6" tall	Low growing, drought tolerant
Creeping mazus	*Mazus reptans*	2" tall	Low-growing with blue flowers in late spring; spreads indefinitely
Fragrant sumac "Gro-Low"	*Rhus aromatica*	20" tall 6–7' spread	Glossy green foliage, turns orange–red in autumn
Heath aster "Schneegitter"	*Aster ericoides*	4–8" tall	White blooms in mounds
Lady's mantle	*Alchemilla mollis*	8–16" tall 12" spread	Attractive green foliage and cream inflorescence
Lamb's ear	*Stachys byzantina*	12–18" tall	Silver foliage, inflorescences easily lodge
Maiden Pinks "Brilliant"	*Dianthus deltoides*	8" tall 16" spread	Evergreen with deep rose-colored flowers
Ornamental Goldenrod	*Solidago sphacelata*	4–8" tall	Attractive yellow flowers, drought and salt tolerant
Fleeceflower	*Persicaria affinis*	5–8" tall	Foliage becomes colorful in fall, attractive pink–red poker-shaped flowers
Sedum	*Sedum acre*	<3" tall	Low-growing green to blue green succulent foliage, drought and stress tolerant

Fig. 1. Examples of selected weed suppressive groundcovers which are also aesthetically attractive in various landscape settings. They include catmint (**a**, *Nepeta x faasennii*), Japanese anemone (**b**, *Aneomone x hyrbrida*), aurenia (**c**, *Aurenia saxatilis*) and laurentia (**d**, *Laurentia fluviatilis*)

18.3 Weed Suppressive Turfgrasses as Groundcovers

An effective preventive strategy to minimize weed infestation in turfgrass or roadside settings also involves the use of appropriate turf mixtures or cultivars that are well adapted to a given setting for optimal density and growth. Weeds are much less likely to invade a dense turf in good condition, maintained with appropriate cultural practices including timely mowing, overseeding and fertilization (Turgeon 1999). Due to the increased interest in establishment of aesthetically appealing, low maintenance landscapes, our research has focused on the selection and utilization of certain turfgrasses as low maintenance, stress tolerant and weed suppressive for landscape and roadside settings (Bertin and Weston 2004, 2006).

The genus *Festuca* represents one of the largest groups of grasses in the tribe Poaceae (Clayton and Renvoize 1986). Approximately 100 different fescue species are currently found in the United States and Europe. If one looks closely at a collection of fescues, it is easy to see that *Festuca* species vary greatly in morphology and growth habit. Generally, the fescues are divided by appearance and usage patterns into two specific types: the fine or coarse leaf fescues. Fine-leaf fescues are among the most common turfgrass species used in the northern and central United.States and Europe for lawns and turf, especially in low maintenance settings with poor soils or shaded sites. The fine-leaf fescues include slender and strong creeping red (*F. rubra ssp. rubra* and *F. rubra* ssp. trichophylla or ssp. *littoralis*), chewing's fescues (*F. rubra ssp. commutata*), hard fescues (*F. longifolia*) and sheep fescues (*F. ovina*). These five types of cool season fine-leaf fescues are commonly used as not only as turfgrasses but also for forage, turf or conservation purposes (Bertin et al. 2007; Jauhar 1997).

The turf-type fine-leaf fescues, specifically red, chewings and hard fescues, have been recently selected for improved disease and heat resistance, as well as darker color and ease of establishment (Ruemmele et al. 1995). These grasses prefer to be established in well-drained soils, and are tolerant of both full sun and shad. They prefer a non-alkaline soil, with lower soil pH. In general, the fine-leaf fescues do not require much fertilization or irrigation, unless under exceptionally stressful growing conditions. The root systems of most fine-leaf fescues tend to be shallow and fibrous, making them less acceptable for use in sports fields and high traffic areas in comparison to their tall fescue relatives. In high heat and drought conditions of late summer, the fine-leaf fescues often enter a dormant state and turn brown. They have not died, but rather when cooler moist conditions return in the fall, they will generate root growth and luxuriant foliar growth as well. In periods of extreme drought such as those encountered in 2005, we did loose establish stands of fine-leaf fescue on particularly well-drained, dry sites and overseeding was necessary in many locations across New York State, but this is generally not routine (Bertin and Weston 2006; www.hort.cornell.edu/department/faculty/weston/fescue/weedsuppressive.html).

In contrast, the tall fescues (*Festuca arundinacea*) are relatively coarse-leaved, darker green grasses that are drought resistant and somewhat shade tolerant in more southern locations. They are well-adapted to heavy clay soils and perform well in the transition zone states with hot summers and cold winters. Endophyte-free tall fescues are used extensively as forage grasses in pastures and rangelands throughout the U.S. Other improved turf cultivars are utilized on roadsides and also more recently as attractive turfgrasses in transition zone areas or as managed turfs on athletic fields. The turf-type tall fescues were selected and extensively bred to improve heat and drought tolerance, endure high traffic, and to have narrower leaves than the pasture-type such as Kentucky-31. Although tall fescue has short rhizomes, it has a limited capacity to spread given its bunch-like growth habit, and can thin in the shade. Reseeding on a regular basis may be needed to retain desired density over time. Compared to fine-leaf fescues, the leaf blades of coarse fescue can be relatively tough and require a sharp blade while mowing to prevent ragged edges. Recommended mowing height for tall fescue is generally 2.5–3 inches to avoid scalping and maintain density, while fine fescue can be successfully mowed and maintained at lower heights (Bertin and Weston 2006; www.hort.cornell.edu/department/faculty/weston/fescue/weedsuppressive.html).

Fine-leaf fescues can also be very attractive when maintained without mowing for a natural look, and create a wave-like effect of blueish, green ripples across the landscape. We see them often used *au naturel* in golf course roughs, European golf courses, sloped sites and reclamation areas (Morgan and Boubaki 1999). Like fine-leaf fescue, tall fescue tolerates periods of drought, but in high temperatures, tends to go dormant. Both fine- and coarse-leaf fescues are susceptible to several diseases that may be exacerbated by exposure to excessive irrigation and fertilization. Fine fescues are susceptible to common diseases including red thread and dollar spot, while tall fescue is susceptible to brown patch and fescue leaf spot (Bertin et al. 2008; Bertin and Weston 2006; Ruemmele et al. 1995).

Recently, fescue breeders have developed cultivars that possess tolerance not only to acidic soils and those with low fertility, but also to a variety of sun exposures including substantial shade as well as full sun. Although fescues are often slower to germinate and establish than other turfgrasses, such as perennial ryegrass, newer cultivars have been selected for more rapid establishment and green-up (Ruemmele et al. 1995). When fall seeded at recommended rates for turfgrass stands, these newer cultivars such as Oxford, Intrigue, Sandpiper and Rescue 911, typically establish a dense, weed-free turf by July of the following year. Currently, there is increased interest by the U.S. and European turfgrass industries in the utilization of fine leaf fescues for both lawn and golf turf as well as for low maintenance settings with exposure to stressful conditions, including cold temperatures, drought and saline soil conditions or roadside salt spray (Bertin et al. 2008).

Over the last decade, the study of plant–plant interactions and utilization of allelopathy and plant interference as a potential weed management tool has received increasing attention. The use of allelopathy for weed management relies upon the species-specific responses of a target weed to chronic, and/or sublethal doses of an allelochemical (plant growth inhibitor), which can be exuded or leached from nearby living plants or decomposing residues (Putnam and Tang 1986). Weed suppressive cover crops that have been successfully used to suppress annual weeds have included economically important cereals such as wheat (*Triticum aestivum*), oat (*Avena sativa*), rye (*Secale cereale*), barley (*Hordeum vulgare*), sorghum (*Sorghum bicolor*) and rice (*Oryza sativa*) (Weston 1990, 1996; Wu et al. 1999). Although studies on allelopathic crops have focused on these key species, many other weedy and crop species show promise of allelopathic potential for suppression of surrounding vegetation, including several turfgrasses such as buffalo grass, perennial ryegrass, Bermuda grass and both tall and fine-leaf fescues (Weston and Duke 2003; Weston 2005). However, until recently, few studies have been conducted to further evaluate the weed suppressive potential of these species. Our recent trials with weed suppressive turfgrasses have shown that the ability to establish rapidly, produce a dense turf or canopy thereby reducing light availability at the soil surface, and the subsequent allelopathic properties of an established turfgrass can positively influence the long-term ability of a turfgrass to result in near complete suppression of typical turf weeds, including large crabgrass (*Digitaria sanguinalis*) and annual bluegrass (*Poa annua*), if routine mowing is performed without scalping of the turf (Bertin et al. 2008).

A review of the literature shows that *Festuca* spp. can be strongly weed suppressive when used for erosion control in agronomic, orchard and vineyard settings (Malik et al. 2000; McGourty and Christensen 1998). Prior studies have focused on the weed suppressive effects of tall fescue, which was shown to be potentially allelopathic by production of toxic root leachates (Bertin et al. 2003; Peters and Zan 1981). In 1990, in studies in Kentucky, we demonstrated that creeping red fescue (*F. rubra spp. rubra* and ssp. *tricolphylla*) was highly weed suppressive when established as a living mulch or as killed sod in no-tillage field experiments (Weston 1990). By conducting a series of field studies across New York State, we have demonstrated that certain cultivars of creeping red, chewings or hard fescue exhibit the ability to effectively suppress weeds over a multi-year period. An initial study was conducted in 1999–2002 as part of the National Turfgrass Evaluation Program (NTEP) to evaluate a collection of 78 fine-leaf fescue cultivars for turfgrass quality, seedling vigor, and ability to suppress the establishment of common annual and perennial weeds. Using these criteria the overall suitability of the cultivars for use in turfgrass settings was evaluated, along with their potential to inhibit the establishment of common turf weeds. A few cultivars appeared to be standouts in terms of turf quality and provided good to very good suppression of common turf weeds, while other cultivars proved to be moderately or poorly weed suppressive over time (Bertin et al. 2008).

In related laboratory research, we observed that greater weed suppression was likely associated with the differential ability of fescue cultivars to establish rapidly and maintain a dense turf, as well as their potential to exhibit allelopathic interference (Fig. 2). Certain fine-leaf fescue cultivars exhibited greater ability to suppress weeds in agar or sand culture, through production of large quantities of inhibitory root exudates from fine fibrous fescue roots (Bertin et al. 2003). Other cultivars, which were less suppressive in field experiments, were also less suppressive to weed growth in the laboratory. Interestingly, fescue roots cultured under simulated drought conditions produced up to three times greater levels of weed suppressive root exudates than did those produced under normal growth conditions, indicating that a slight stress may result in enhanced allelochemical production (Bertin and Weston 2006; Bertin et al. 2007).

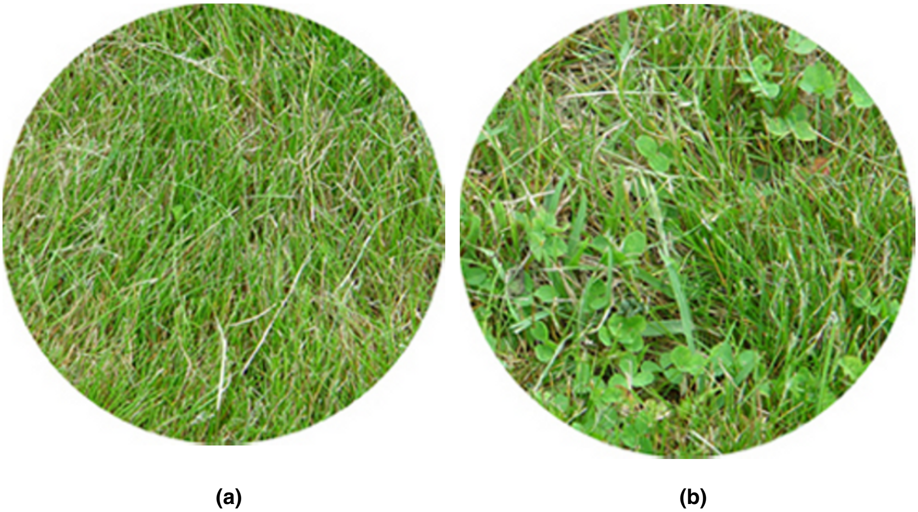
(a) (b)

Fig. 2. An example of a weed suppressive fine fescue (**a**, Intrigue) and a non-weed suppressive fine fescue (**b**, Attila)

In additional field studies conducted across New England with a selection of both weed suppressive and non-suppressive cultivars, and using a large number of repetitions or replicates for increased precision, the fine-leaf fescue cultivars Intrigue, Columbra, and Sandpiper proved to be excellent performers in terms of weed suppression and turf appearance (less than 5–8% weed infestations) over time than other fine-leaf fescue cultivars. Reliant II, Wilma and Oxford were also very good performers in terms of weed suppression, while Treasure, Boreal, Rebel II tall fescue, Sylvia High and several other numbered selections were much less suppressive (greater than 15–30% infestations). All cultivars were established at a standard seeding rate. Although our field studies show that certain fescue cultivars clearly possess differential ability to suppress weeds over time, further studies are required to improve our understanding of the factors influencing this weed suppression. For instance, we would like to determine what factors clearly impact the production of weed suppressive root exudates under field conditions (Bertin and Weston 2006; www.hort.cornell.edu/department/faculty/weston/fescue/weedsuppressive.html).

Most recently, we isolated and identified one key bioactive constituent in the inhibitory root exudates collected from weed suppressive chewings fescue (Bertin et al. 2007). This highly active inhibitor was identified as m-tyrosine, a simple derivative of the common amino acid tyrosine (Bertin et al. 2005). In laboratory experiments, m-tyrosine was a potent inhibitor of weed seedling root growth and germination whereas other tyrosine analogues were not inhibitory. Seedling growth of all common turf and crop weeds were strongly impacted by m-tyrosine. M-Tyrosine was found in large quantities in root exudates of chewings fescue cultivars, strong red creeping cultivars and Arizona fescue (*Festuca arizonica*). When tested upon the growth of mammalian cell cultures, the chemical did not exhibit negative impacts upon cell growth. It may have potential to be developed for use as a pre-emergent soil herbicide, as it is soil active at rates similar to those of several preemergent turf herbicides, such as pendimethalin. It appears that the compound definitively impacts cell division and cell elongation in higher plant species, but we are currently attempting to identify its specific mode of action, and determine, with industry support, its potential to be developed as a soil-applied natural herbicide (Bertin et al. 2005; Bertin and Weston 2006).

In another series of recent studies, we evaluated a diverse collection of over 25 turfgrass species and cultivars for their ability to establish across the Northeast in a variety of field and roadside settings (unpublished data). Our better *Festuca* performers have continued to establish well and provide great weed suppression. In addition, Russian wild rye (*Psathrostachys juncea*), redtop (*Agrostis stolonifera*), and perennial ryegrass (*Lolium perenne*) cv. Palmer IV also provided excellent cover and good weed suppression one year following establishment. For use along roadsides, the ultimate objective is to select a turfgrass cultivar or mixture that is tolerant of drought, salinity, low fertility, requires limited mowing, and establishes successfully such that it is highly weed suppressive over time. A daunting task? Perhaps, but continued collaboration with plant breeding specialists indicates that we have potential to select for enhanced weed suppression and stress tolerance among existing turfgrass and ornamental species as considerable genotypic diversity exists in germplasm collections. Although breeding for highly weed suppressive groundcovers is a novel concept, the development of low maintenance plant materials, including turfgrass, with enhanced stress tolerance is not. The appropriate combination of these value-added attributes will hopefully lead to the future development and release of new cultivars and mixtures of species which have utility as weed suppressive groundcovers in low maintenance settings (Table 2) (Weston 2006; www.hort.cornell.edu/deparment/faculty/weston/grassandforbmixes/weedsuppressive.html).

Table 2. Certain fine fescue cultivars which have exhibited variable performance in trials across New York State, in terms of weed suppression and turf quality. Others evaluated (over 60 cultivars) were ranked generally as moderate performers

Fine fescue cultivar	Fescue type	Seeding rate	Performance
1. Intrigue	chewings	4lb/1000 sq ft	Excellent
2. Columbra	chewings	4lb/1000 sq ft	Excellent
3. Wilma	chewings`	4lb/1000 sq ft	Excellent
4. Reliant II	hard	4lb/1000 sq ft	Excellent
6. Sandpiper	chewings	4lb/1000 sq ft	Very good
7. Oxford	hard	4lb/1000 sq ft	Very good
8. Rescue 911	hard	4lb/1000 sq ft	Very good
9. Treasure	chewings	4lb/1000 sq ft	Poor
10. Atilla	hard	4lb/1000 sq ft	Poor
11. Sylvia high	hard	4lb/1000 sq ft	Poor

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19. Allelopathy in Forested Ecosystems

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Abstract. In the context of forestry, the concept of allelopathy has recently been expanded from a plant to plant interference phenomenon to an ecosystem-level phenomenon that is influenced by ecosystem disturbance. This chapter reviews the latest development in our understanding of forest allelopathy and the ways in which this new knowledge can be used in sustainable forest management. Allelopathic effects of certain canopy trees on tree seedlings and understory plants have direct effects on forest renewal. Likewise some understory plants with allelopathic property can have controlling effects on tree regeneration and species composition. In fire adapted boreal forests, particularly natural fires, the level and distribution of fire severity plays a critical role in the manifestation of forest allelopathy. The high severity fires break down allelochemicals by thermal decomposition, create favorable seedbed by consuming forest floor humus and releasing nutrients and removing competing plants by killing underground regenerating organs. Clearcut harvesting and low-severity fires on the other hand, may promote vegetative regeneration of understory plants with competitive and allelopathic properties as their underground perennating structures remain unharmed. This may cause retrogressive succession by resisting tree colonization and inducing long-term habitat degradation. Research in the last two decades has increased our understanding on the mechanism of forest allelopathy in fire adapted boreal forests, but our knowledge in forest allelopathy in tropical forest is very limited. Control of competing and allelopathic plants after forest harvesting is a serious issue in forestry and public opinion is not favorable in using chemical herbicides. Alternative methods such as use of allelopathic straw mulch, herbicides of biological origin (bialaphose), planting tree seedling pre-inoculated with mycorrhiza, and scarification and spot fertilization at planting have produced good results. It is possible to develop alternative methods of weed control in forestry by using allelopathy principle. However, substantial infusion of research and development funds is necessary to make significant progress in this area.

19.1 Introduction

Although the study of allelopathy involving plants in agricultural and horticultural systems has a long history, allelopathy research in forested ecosystems is rather recent. Unlike the modern simplified agricultural systems where the desired crops (free from weeds) are grown seasonally in monocultures on cultivated homogenized land, forested ecosystems are much more complex both temporally and spatially with high habitat heterogeneity and species diversity. In forested ecosystems allelopathic effects can be induced by canopy trees on conspecifics (by auto-toxicity) as well as on other tree species causing forest decline. Trees and understory plants can also affect each other allelopathically causing an overall decline in species richness and diversity. These changes happen at varying spatial and temporal scales causing local to ecosystem-level differences in forest structure and composition. Changes in forest structure and composition can also be induced by the so called 'afterlife effects' of humus and their decomposition products, some of which are inhibitory to germination and seedling growth. The adverse allelopathic effects of canopy species such as walnut trees (*Juglans* spp.) on other plants in their vicinity through volatile emission of juglon, a napthoquinone is one of the earliest reported cases of forest alleopathy (Davis 1928). In the first AD Pliny the Elder wrote in his Naturalis Historia "the shade of the walnut even caused headaches in man and injury to anything planted in the vicinity". Similar observations were made by other authors during the Middle Ages to modern times. The American species, black walnut (*J. nigra*), has been known to have injurious effects on other plants including crops (Jose and Holzmueller, this volume). Allelopathic effects of tree species such as *Eucalyptus* spp., *Acacia* spp., *Albizzia labbeck, Populus deltoids* are known to affect other plants in natural forests, plantations and agricultural fields. Dramatic declines in understory species abundance and diversity, forest floor organic matter depletion, soil erosion and habitat degradation and reduction in crop production have been attributed to allelopathy in many parts of the world (Lovett, 1989; May and Ash, 1990; Carballeira and Reigosa, 1999; Casal, Reigosa and Carballeira, 1985; Singh and Kohli, 1992).

Since the increase in agricultural productivity has been a human preoccupation since the first domestication of plants as a crop, it is not surprising that disproportionately large number of allelopathy research has been concentrated on agricultural crops and weeds compared to studies in forest allelopathy. We find extensive writings on chemical interference of crops and weeds in Greek and Roman agricultural treaties (Aliotta, Mallik and Pollio, 2007). In agricultural systems allelopathy studies have been approached from the point of view of plant–plant interaction, although soil sickness involving crops has been an issue recognized and dealt within various ways since the early days of agriculture and horticulture. This was also the case with earlier studies of tree species allelopathy. However, the recent approach in forest allelopathy is far more complex, involving multiple life-forms and abiotic factors in the ecosystems including disturbance. The earlier notion of plant–plant interaction has been expanded temporally from the annual cropping cycles to centuries of natural succession and spatially extending from localized to ecosystem level and organizationally from population to community level. Inclusion of natural and anthropogenic disturbance into the design of allelopathy studies makes it even more challenging, albeit more realistic (Wardle, Nilsson, Gallet and Zackrisson, 1998). Thus the notion of allelopathy being a localized neighborhood effect of plant-plant interference has been extended to a broader landscape-level ecological phenomenon (Fig. 1).

Recently, convincing arguments have been made by demonstrating allelopathic effects of understory plants causing ecosystem level change by inducing habitat modification and controlling regeneration capacity of canopy trees in natural and managed ecosystems (Wardle et al., 1998; Nilsson and Wardle, 2005; Mallik, 2003).

This has implications for both fundamental understanding of plant species interactions as well as large-scale anthropogenic management of forested ecosystems.

The objective of this chapter is essentially three fold: (i) to briefly review what we know of allelopathy in natural and managed forested ecosystems at stand and ecosystem levels, (ii) to discuss to what extent and by what means allelopathy and soil chemical ecology control forest community structure and composition and (iii) to explore if this knowledge can be used in devising sustainable forest management practice and biodiversity conservation.

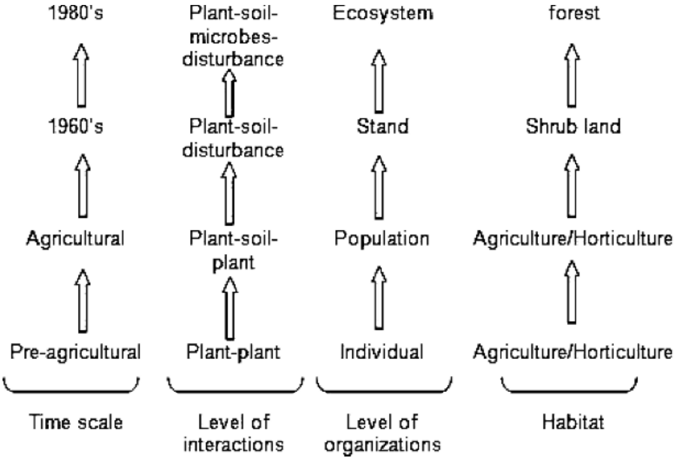

Fig. 1. Advances in the understanding of allelopathy over time with increasing complexity

Many forest plants can have allelopathic effect on each other. Trees being the dominant and commercially valuable life-form, most attention have been paid to their well being and so is the case with crop plants in agricultural systems. As in an agricultural system, we observe allelopathic effects of trees on conspecifics (autotoxicity) and in other life-forms of successively lower stature such as shrubs, herbs and lichens. Many of the understory plants can also have allelopathic effects on tree species particularly when the trees are at the seedling stage (Pellissier, Gallet and Souto, 2002).

19.2 Canopy Species Effects on Tree Seedlings and Understory Plants

The most common effect of canopy trees on understory plants is through competition whereby the former deprive the latter from environmental (light, temperature etc.) and soil resources (water, nutrients etc.). However, many canopy tree species can also affect their own seedlings and the understory plants allelopathically (Table 1).

It is often difficult to distinguish experimentally the contributions of the individual mechanism (e.g. competition, allelopathy and symbiosis) involved as they often operate interactively and simultaneously (Inderjit and del Moral, 1997). By far the majority of studies in allelopathy cited in the literature are conducted under controlled conditions and very few considered the interacting factors at play under natural conditions (Tables 1 and 2). Nonetheless, some tree species provide clear evidence of allelopathic effect, for example black walnut.

Fast growing *Eucalypus* and *Acacia* plantations in Europe and Asia are good examples of this type of allelopathic interference (Reigosa, Casal and Carballeira, 1984; Carballeira and Reigosa, 1999; Lothi and Rice, 1971). High phenolic contents of litter under mature conifers such as Norway spruce (*Picea abies*) have been attributed to allelopathic inhibition of seed germination and seedling growth of the conifer (Gallet, 1994). Allelochemicals derived from living, dead and decomposed tissues of the canopy trees can not only interfere with tree regeneration directly by affecting seed germination and seedling growth (Nilsson and Zackrisson, 1992; Pellissier, 1993), but also indirectly by interfering mycorrhizal symbiosis (Mallik, Zhu and Park, 1998; Yamasaki, Fyles, Egger and Titus, 1998). Kil (1992) reported sparse understory flora beneath three pine canopies, Korean red pine (*Pinus densiflora*), pitch pine (*P. rigida*) and black pine (*P. thunbergii*). He also found that the understory floristic composition was different between the pine and non-pine stands (Lee and Monshi, 1963; Kil and Yim, 1983). Kil (1992) hypothesized that the floristic difference was due to some understory regeneration mechanisms controlled by pine through release of allelochemicals in soil. The author demonstrated that growth of understory plants was inhibited in soil mixed with pine needles; however, over time the growth inhibition disappeared. He showed that aquatic extracts of fresh needles inhibited seed germination more than that of the dry needles, and black pine needles were most inhibitory compared to the other two pine species. Kil (1992) also compared the biomass of pine and non-pine forest understory plants after growing them reciprocally in pine and non-pine soils. The growth response was mixed, some plants grew better in their own soil, some were indifferent and yet others were sensitive to pine soil. Several water soluble phenolic acids were attributed to the germination and seedling growth inhibition. However, to determine the influence of canopy trees on understory regeneration through chemical interference more careful experiments are required by eliminating the effects of other soil ecological factors involving microorganisms.

In addition to affecting the understory plants directly, the allelochemicals of the canopy species can influence understory species indirectly by affecting their symbiotic relationships with microorganisms, the rhizosphere ecology and nutrient availability. Using a soil percolation apparatus, Thibault, Fortin and Smirnoff (1982) demonstrated that 5% water extract of balsam fir (*Abies balsamea*) needles completely prevented oxidation of ammonium. Similarly, Lodhi and Killingbeck (1980) observed allelopathic inhibition of nitrification in a ponderosa pine (*Pinus ponderosa*) forest. Jobidon (1992) reported that degraded forest sites of Quebec were often colonized by alders (*Alnus* spp.), the most common nitrogen fixing understory plants which were then replaced by poplar species (*Populus tremuloides* and *Populus balsamifera*). The frequently observed growth reduction of alder near newly established balsam poplar was studied by Thibault et al. (1982) from the point of view of allelopathy of the canopy trees. They found that foliage leachate of balsam poplar significantly inhibited seed germination and seedling growth of green alder (*Alnus regosa*). The affected seedlings showed poor root hair development and necrosis of the radicle. These authors suggested that the lack of root hair formation may have direct effect on harboring the nitrogen fixing actinomycete, *Frankia* spp. on root since the endophyte must penetrate through the root hairs to initiate nodulation (Lalonde and Quispel, 1977; Lalonde, 1979; Callaham, Newcomb, Torrey and Peterson, 1979). The subject was further studied by Jobidon and Thibault (1982) who showed that water extracts of different aerial parts of balsam poplar caused significant inhibition of growth, nodulation and nitrogen fixation in green alder (Perradin, Mottet and Lalonde, 1983) tested the effects of several plant phenolics (some are common in balsam poplar) on in vitro growth of six *Frankia* strains. They found that cinnamic acids particularly, o-coumaric, p-coumaric and ferulic acids are strongly inhibitory to colony growth of *Frankia* spp. In this case the developmental impairment of roots due to allelopathy caused a cascading effect by inhibiting actinomycete infection and consequently poor growth due to N limitation. Their results suggest that succession from a shrub dominated alder community to a tree dominated balsam poplar community may be controlled by the direct and indirect allelopathic effects of the canopy tree. Many phenolic allelochemicals can indirectly affect tree seedling growth by interfering tree-microbe symbiotic relationships and soil nutrient availability. Several authors reported high concentration of coumaric acid as well as other phenolic allelochemicals in Norway spruce humus that are known to interfere with seed germination and seedling growth of the conifer (Pellissier, 1993, 1994; Gallet, 1994; Gallet and Lebreton, 1994). Some of these phenolic compounds can also affect the growth of conifer ectomycorrhizae by increased respiration (Boufalis and Pellissier, 1994). Seasonal changes in accumulation of these humus allelochemicals interfere with other soil microbes directly associated with nutrient cycling (Souto, Chiapusio and Pellissier, 1998).

Under natural forest conditions coexistence of conifers with understory plants makes it difficult to separate the effect of one from the other. However, controlled experiments with different humus types can provide convincing evidence of allelopathy. Although often challenging, demonstration of canopy tree allelopathy in regulating understory community structure and composition is possible by careful experiments that eliminate other possibilities. One must attempt to test for alternative hypotheses. For example red pine litter leachate has been known to affect understory plants chemically, but sheer large accumulations of dry pine litter on forest floor can smother understory plants, create inhospitable substrate for germination and seedling growth by creating a physical barrier for primary roots to reach mineral soil for moisture and nutrient (Siegwart and Mallik, 2007, unpublished data).

Table 1. Allelopathic effects of tree species on tree seedlings, understory herbs, shrubs and model plants (modified from Pellissier et al., 2002)

Allelopathic species	Target species	Affected physiological process	Allelopathic agent or chemicals	Experiment	Country	Reference
A. Conifer	Old field weeds:					
Picea mariana *Pinus resinosa* *Pinus divaricata* *Thuja occidentalis* *Abies balsamea*	*Poa pratensis* *Agropuron repens* *Epilobium angustifolium* *Phelum pretense* *Picea mariana* (autoinhibition)	Germination, radicle growth	Water-soluble chemicals, foliage lechate	In vitro	Quebec, Canada	Jobidon (1991b); Mallik and Newton (1988)
	Herbaceous plants e.g.:					
Pinus densiflora *Pinus thunbergii* *Pinus rigida*	*Arundinella hirta* *Erigeron annus* *Leonurus sibiricus* *Rumex japonica* *Lecuta sativa* *Perilla frutesceus* *Matricaria chamomilla*	Primary growth of seedlings	Needle and root phenolics	In vitro	Korea	Kil and Yim (1983); Kil (1992); Chou et al. (1989)
Abies alba	*Lepidium sativum*	Germination, radicle growth	Water-soluble chemicals (phenolics)	In vitro	N. France	Becker and Drapier (1984, 1985)
Picea abies	*Picea abies* (autoinhibition)	Germination inhibitor	p-hydroxy-acetaphenone		S. France	Gallet (1994)

Allelopathic species	Target species	Affected physiological process	Allelopathic agent or chemicals	Experiment	Country	Reference
B. Decidous						
Juglans nigra	*Surrounding species*		Juglone	In vitro (?)		Davis (1928)
Eucalyptus globulus	*Lactuca sativa*	Germination, radicle growth	Decaying litter	In vitro	Spain, Australia	Lovett, 1989 Souto et al. (1994)
Acacia spp.	*Understory plants*			In vitro	Spain	Casal et al., 1985; Carbarella and Reigosa, 1987
Acer circinatum *Acer saccharum* *Sambus racemosa*	*Pseudotsuga mengziessi*	Radicle growth	Water-soluble chemicals	In vitro	USA	Tubbs (1973)
Quercus robur *Fagus sylvatica*	*Scrophularia nodosa* *Senacio sylavaticus* *Millium effusum* *Holcus lanatus*	Seedling growth, chlorophyll content	Polyphenols, tannins	In vitro	Europe	Kuiters (1987)
Poplus balsamifera *Prosopis juliflora* *P. cineraria*	*Alnus crispa*	Seedling growth, radicle growth, nodulation	Water-soluble chemicals	In vitro	USA, Canada	Jobidon and Thibault (1982); Goel et al. (1989)

Table 2. Selected examples of allelopathic effects of understory herbs and shrubs on tree species (modified from Pelliessier et al., 2002)

Allelopathic species	Target species	Affected physiological process	Allelopathic agent or chemicals	Experiment	Country	Reference
Solidago canadensis *Solidago germinifolia* *Aster nova-angliae*	*Acer saccharum*	Germination, growth	Water-soluble chemicals	In vitro	Canada	Jobidon and Thibault, 1982
Eupatorium capillifolium *Lyonia lucida*	*Pinus elliotti* *Pinus taeda*	Germination, growth	Water-soluble chemicals	In vitro	South USA	Freguson and Boyd, 1988; Horsley, 1977
Pterdium aquilinum	*Pinus syslvestris* *Picea abies* *Populus tremula*	Germination, radicle growth	Volatiles and humus	In vitro, in situ	Sweden	Dolling, 1996
Vaccinium myrtillus *Athyrium filix-femina*	*Picea abies*	Germination, growth	polyphenols	In vitro	European Alps	Pellissier, 1993, 1994; Mallik and Pellissier, 2000
Kalmia angustifolia	*Picea mariana* *Abies balsamifera* *Pinus resinosa*	Germination, root development	Water-soluble chemicals	In vitro	Canada	Mallik, 1987; Thompson and Mallik, 1989; Mallik and Roberts, 1994; Wollenweber and Kohorst, 1994
Calluna vulgaris	*Pinus sylvestris*	Root growth	Water soluble compounds	In vitro	UK	Robinson, 1972
Empetrum hemaphroditum	*Pinus sylvestris* *Betula spp.*	Growth	Water-soluble chemicals, phenols, batatasin	In vitro, in situ	Sweden	Oden et al., 1992

19.3 Understory Effects on Tree Seedlings

Many understory plants have been reported to have inhibitory effects on seed germination and seedling growth of canopy trees (Pellissier et al., 2002). Once again although competition plays a major role in tree-understory interaction, allelopathic interference also plays a significant role, particularly in temperate forests. Pellissier and Souto (1999) have reviewed the role of allelopathy in semi-natural temperate forests of Western Europe. They concluded that in both conifer and deciduous forests allelopathic effects of understory plants and forest floor humus can directly affect tree regeneration. They suggest that forest allelopathy involves soil microbial community, particularly mycorrhizal fungi and other microorganisms that act as allelochemical mediators by activating and inactivating soil organic compounds by metabolic activities. Most studies on allelopathic effects of understory plant on tree species are conducted in controlled conditions and extrapolation of results to field conditions is difficult (Table 2).

The species poor boreal forests have three distinct vegetation strata; the uppermost tree canopy consisting of mostly conifers and a few deciduous hardwood species, the intermediate shrub layer often dominated by ericaceous plants and the ground layer dominated by mosses, lichens and creeping herbs and shrubs (Nilsson and Wardle, 2005). Although the species complements of the three vegetation layers may vary depending on geographic location, natural and anthropogenic disturbances (such as forest harvesting and silviculture), the competitive and potential allelopathic effects of the understory shrubs on canopy tree regeneration can be quite similar. For example, Sitka spruce (*Picea sitchensis*) growth inhibition in Britain in the presence of heather (*Calluna vulgaris*, hereafter referred to as *Calluna*) was reported as early as 1953 (Weatherell, 1953; Leyton, 1954, 1955; Handley, 1963). This has been attributed to direct allelochemical interference of *Calluna* root exudates on tree species and indirectly via ectomycorrhizal inhibition and nutrient lock-up (Robinson, 1971, 1972; Read and Jalal, 1980; Jalal and Read 1983a,b; Bending and Read, 1996a,b). Similarly, failure of natural regeneration and growth inhibition of planted conifers such as black spruce in eastern Canada in the presence of sheep laurel (*Kalmia angustifolia* hereafter referred to as *Kalmia*), blueberry (*Vaccinium angustifolium*) and Labrador tea (*Ledum groenlandicum*) was attributed to combined effects of resource competition and allelopathy (Mallik, 1987, 1992; Inderjit and Mallik, 1996a; Yamasaki et al., 1998; Yamasaki, Fyles and Titus, 2002; Thiffault, Titus and Munson, 2005). Growth inhibition of jack pine (*Pinus banksiana*) in the presence of *Kalmia* has been reported from New Brunswick, Canada by Krause (1986).

Most of the ericaceous litter contains an array of phenolic compounds that are inhibitory to conifer seed germination, primary root growth and ectomycorrhizal growth (Facelli and Pickett, 1991; Pellissier, 1993, 1994; Gallet and Lebreton, 1995; Mallik, 1987, 1992; Zhu and Mallik, 1994; Mallik and Zhu, 1995; Mallik et al., 1998; Mallik and Pellissier, 2000). Many of these phenolic compounds can create soil nutrient imbalance by reducing available N and increasing the amounts of Fe, Zn, K, Ca, Mg and Mg leading to long-term site degradation (Bending and Read, 1996a, b; Damman, 1971, 1975; Meades, 1983, 1986; Inderjit and Mallik, 1996a,b, 1997a,b). From the west coast of Canada and the Pacific Northwest of the USA other ericaceous shrubs such as salal (*Gaultheria shallon*) in coastal oceanic temperate rainforests and several *Vaccinium* species (particularly *Vaccinium alaskaense*) in high elevation forests have been reported to cause growth stagnation of conifers such as western red cedar (*Tsuga plicata*), western hemlock (*Thuja heterophylla*), Sitka spruce (*Picea sitchensis*) and Amabilis fir (*Abies amabilis*) (Bunnell, 1990; Messier, 1993; Fraser, 1993; Prescott, Weetman and Baker, 1996; Fraser, Turkington, and Chanway, 1993; Fraser, Chanway, and Turkington, 1995).

Belowground competition for space, available N and P and to some extent condensed tannin allelopathy of salal litter have been suggested as the principal causes of growth check in these conifers (Taylor and Tabbush, 1990; Prescott et al., 1996; Xiao and Berch, 1992; de Montigny, 1992; de Montigny and Weetman, 1990; Mallik and Prescott, 2001). In the sub-alpine spruce forests of southern France, yet another understory ericaceous plant, bilberry (*Vaccinium myrtillus*) has been reported to cause regeneration failure of Norway spruce (*Picea abies*) (Maubon, Ponge and Andre, 1995; Jaderlund, Zackrisson and Nilsson, 1996; Jaderlund, Zackrisson, Dahlbaerg and Nilsson, 1997). Phenolic allelochemicals of forest floor humus and seed predation have been implicated for this regeneration failure (Pellissier, 1994; Gallet and Lebreton, 1995; Gallet, Nilsson and Zackrisson, 1999).

In northern Sweden black crowberry (*Empetrum hermaphroditum*) forms thick ground cover and interferes with Norway spruce regeneration (Steijlen and Zackrisson, 1987; Wallstedt, 1998; Zackrisson and Nilsson, 1992; Nilsson, 1994). Large quantities of batatasin, a complex phenolic compound found in the leaves and litter of this understory plant have been attributed to inhibition of seed germination and seedling growth of several boreal tree species (Nilsson and Zackrisson, 1992; Oden, Brandtberg, Andersson, Gref, Zackrisson and Nilsson, 1992; Nilsson, Hogberg, Zackrisson and Fengyou, 1993). Humus and leaf leachates of black crowberry have been found to reduce seed germination and seedling growth inhibition in Scots pine (*Pinus sylvestris*) (Nilsson and Zackrisson, 1992). It also causes reduced nutrient uptake and mycorrhizal infection in other boreal trees. Nilsson and Wardle (2005) suggested that, despite their relatively low contributions to overall standing biomass, the understory shrubs exert significant interference in tree establishment by adding disproportionately large amounts of recalcitrant and phenol-rich litter on the forest floor. Horsley (1977a,b, 1993) was able to demonstrate allelopathic effects of understory plants on tree regeneration by several creative field and complimentary laboratory experiments in the Allegheny forest of northeastern USA that discounted the effect of associated factors other than allelopathy. Several earlier studies have shown that bracken fern (*Pteridium acuilinum*), a common understory plant worldwide, can affect forest regeneration by allelopathic interference (Gliessman, 1976; Ferguson and Boyd, 1988). Compared to the temperate forests, studies in tropical forest allelopathy is rather limited. Chou (1999) reviewed the role of allelopathy in agriculture and forestry in Taiwan. Although claims have been made that many tropical and subtropical herbs, shrubs and trees have allelopathic effects which might be true, most of the studies are based on seed germination bioassays under laboratory conditions and it is difficult to make direct connections with the effects under field conditions. Several field experiments have been reported from Taiwan (Chou, Fu, Li and Wang, 1998; Chou and Leu, 1992). More comprehensive studies on allelopathic effects of tropical plants are desirable.

19.4 Ecosystem Perspectives of Allelopathy Mechanisms

The species-poor conifer-ericaceous communities of the temperate and boreal forests are often characterized by one or two dominant canopy species (often conifers) and one or two dominant understory species (mostly ericaceous). The shrubs in response to canopy removing disturbance grow vigorously and control the post-disturbance community structure and habitat biogeochemistry. Wardle et al. (1998) have argued that in a species poor community allelopathy can have a stronger landscape-level impact compared to a species rich community.

In the early days, allelopathic mechanism was explained by the direct growth inhibitory effect of one plant on the neighboring plants through volatile emissions and soil solutions. This knowledge was used in agriculture and horticulture for selecting and rotating crops to overcome soil sickness and other associated land management issues for sustained yield (Singh, Batish and Kohli, 2001; Zeng and Mallik, this volume). Indirect allelopathic effects of plants involving soil microbial community in the rhizosphere elucidated additional layers of complexity in allelopathic mechanism. Examples of such interactions can be found in agricultural, horticultural and forest plants where allelochemicals have been shown to affect soil microorganisms such as bacteria, actinomycetes and mycorrhizal fungi directly and indirectly associated with plant growth. Furthermore, several authors have demonstrated increased allelopathic activity under microclimatic and soil related stress (Mallik and Bloom 2005; Sánchez-Moreiras, Pedrol, González and Reigosa, 2007). Mallik (1995, 2003) described the possible mechanisms of disturbance induced vegetation shift from forest to ericaceous heath in temperate and boreal forests involving allelopathy and resource competition. He suggests that by linking the concept of ecosystem-level allelopathic effects of dominant species with the concept of organisms as ecosystem engineers (Jones, Lawton and Shachak, 1994), one can explain the long-term ecosystem degradation caused by the engineering effects of dominant plants that have allelopathic properties. The transformation of conifer-ericaceous community to ericaceous heath seems to be a two-stage phenomenon, (i) disturbance mediated removal of canopy trees with concomitant rapid growth of ericaceous shrubs and (ii) ecosystem engineering effects (where allelopathy is a part) of ericaceous plants that on one hand resist tree colonization and on the other hand cause long-term habitat degradation.

Ecosystem-level effects of allelopathy and its interplay with forest fire has been demonstrated quite convincingly in Swedish boreal forests. Working in Swedish boreal forests several authors have shown that forest fires reduce allelopathic effects by breaking down allelochemicals and by adsorbing residual allelochemicals in charcoal (Zackrisson and Nilsson, 1992; Zackrisson, Nilsson and Wardle, 1996; Zackrisson et al., 1997; Wardle, Zackrisson and Nilsson, 1998). Bloom (2001) suggested that site pre-emption by understory shrubs with competitive and allelopathic property such as by *Empetrum* and *Kalmia* following disturbance such as forest harvesting, non-sever fire and large-scale insect defoliation and wind through due to unfavorable seedbed condition for tree regeneration can transform forest into heath.

19.5 Allelopathy in Forest Management

As in agricultural systems weed control by using allelopathy principle can be applied in forest management. However, forestry related research on this subject is rather limited. Broadly three different approaches can be used: (i) application of herbicides of biological origin, (ii) use of allelopathic mulch and (iii) enhancing tree seedling growth by scarification, fertilization and mycorrhizal inoculation.

So far research in developing allelochemicals as herbicides has met with limited success. This is largely because most of the phytotoxins are too weakly allelopathic to be used as herbicides (Inderjit and Keating, 1999). Most commercial herbicides are thousands of times more toxic to weeds than most reported allelochemicals (Duke, Scheffler and Dayan, 2002). Nonetheless, there are several allelochemicals that have potential to be used as herbicides, which needs further research. Sorgoleon obtained from *Sorghum* spp. has strong weed killing property and it works at low concentration by blocking photosystem II of the affected weed (Striebig, Dayan and Rimando, 1999). However, because of the absence of certain desirable physico-chemical properties, its use as herbicide seems difficult (Duke et al., 2002). Work is underway in search of other potent allelochemicals that can be used as herbicides. However, we ought to bear in mind that natural compounds that can be used as herbicides are not always safe since many natural products are potent mammalian toxins and carcinogens such as colchicines, strychnine, alphatoxin and mimosine (Abbas, Boyette and Vesonder, 1993). Of all the phytotoxic chemicals of biological origin, Bialaphos, a microbial metabolic product obtained from *Streptomyces viridochromogenes* produced very good results as a selective herbicide in both agriculture and forestry. It has been discovered in Japan and has been in the market for several years. Bialaphos has been found effective in controlling *Kalmia angustifolia,* and the herbicide does not seem to have any adverse effects on the associated conifers (Jobidon, Thibault and Fortin, 1989a,b; Mallik and Inderjit, 2001).

Use of allelopathic straw mulch to reduce forest weeds has been tried in Quebec, Canada. Jobidon et al. (1989a,b) demonstrated by field experiments that straw mulch of barley, oat and wheat mulch can be used effectively to control red raspberry. While the straw mulches inhibit growth of red raspberry they do not inhibit growth and nutrient concentrations of planted conifers (Figs. 2 and 3). Prescott, Kumi and Weetman (1995) reported that repeated nitrogen fertilizer and straw application in a young jack pine (*Pinus banksiana*) plantation infested with *Kalmia* reduced *Kalmia* cover and improved jack pine growth. Fujii (1999a,b) reported that leguminous cover crops such as hairy vetch (*Vicia hirsuta*) and velvetbean (*Mucuna* spp.) have allelopathic property as wells as nitrogen fixing ability and as such are good candidates for weed control in agriculture.

Another approach may be using ectomycorrhizae inoculated conifer seedlings that can overcome ericaceous allelopathy. Laboratory and greenhouse experiments with conifer seedlings pre-inoculated with selected ectomycorrhiza showed enhanced growth in the presence of *Kalmia* (Mallik et al., 1998). Subsequent research demonstrated that conifer ectomycorrhiza such as *Paccillus involutus* and *Laccaria lacata* can grow well in the presence of *Kalmia* phenolics. They have the ability to break down *Kalmia* phenolics and use them as their carbon source (Zeng and Mallik, 2006). Laboratory experiment demonstrated that *P. involutus* can break down *Kalmia* phenolics such as o-coumaric acid, o-hydroxyphenylacetic acid and ferulic acid in pure culture (Fig. 4). In greenhouse experiment the mycorrhiza inoculated black spruce seedling grown in the presence of *Kalmia* phenolics showed enhanced growth with concomitant reduction in concentrations of these phenolics in the growing medium (Fig. 5).

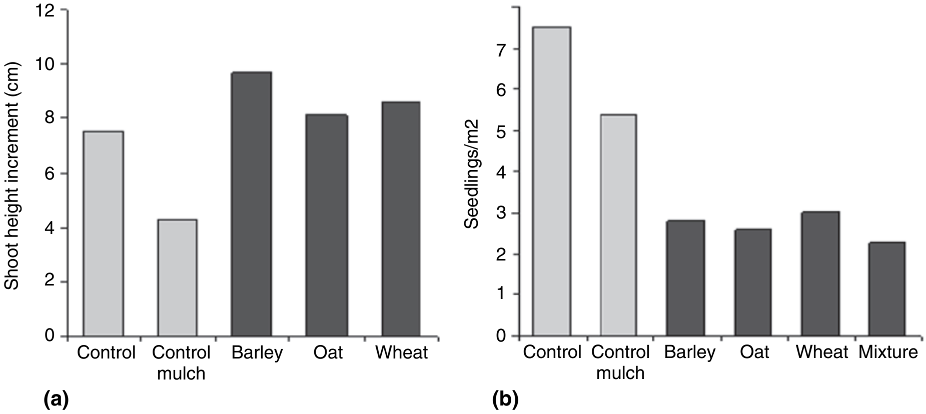

Fig. 2. Shoot height increment (**a**) and number of shoots (**b**) of red raspberry (per m^2) in the second growing season after plowing and barley, oat and wheat straw treatments in young black spruce plantation infested with red rasp berry in Quebec. Data from Jobidon et al. (1989a)

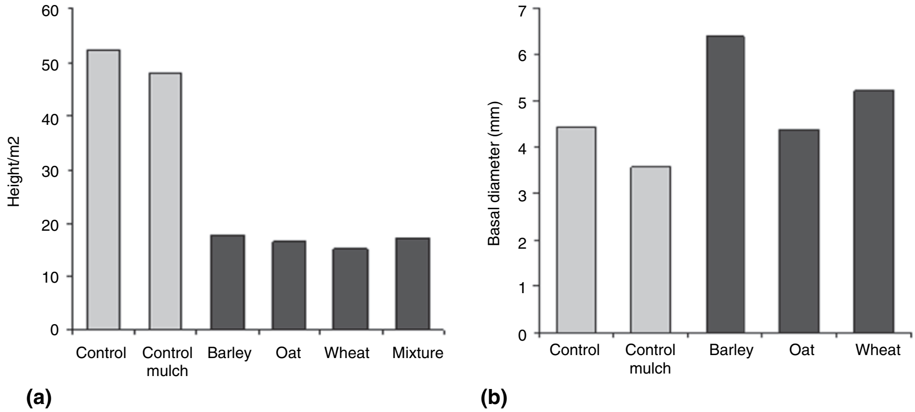

Fig. 3. Mean stem height (**a**) and basal diameter (**b**) of black spruce in the second growing season after plowing and barley, oat and wheat straw treatments in young black spruce plantation infested with red rasp berry in Quebec. Data from Jobidon et al. (1989b)

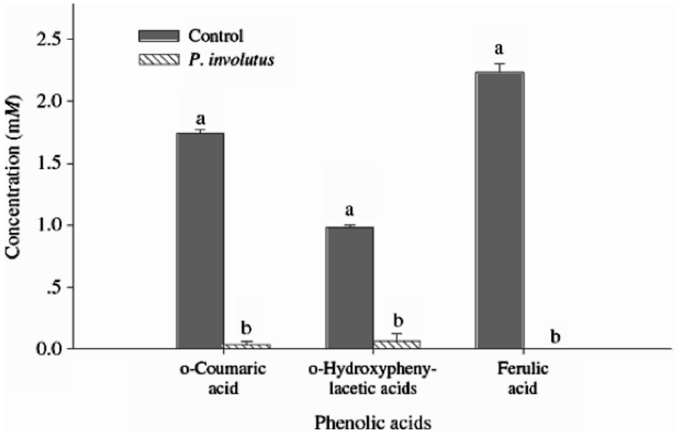

Fig. 4. Degradation of o-coumaric, o-hydroxyphenylacetic and ferulic acid by *P. involutus* innoculated black spruce under greenhouse conditions. The phenolic acids were added in the growing medium. Values are mean + standard error (n = 4). Significant differences among the treatments are indicated by different letters above the histograms (reproduced from Zeng and Mallik, 2006)

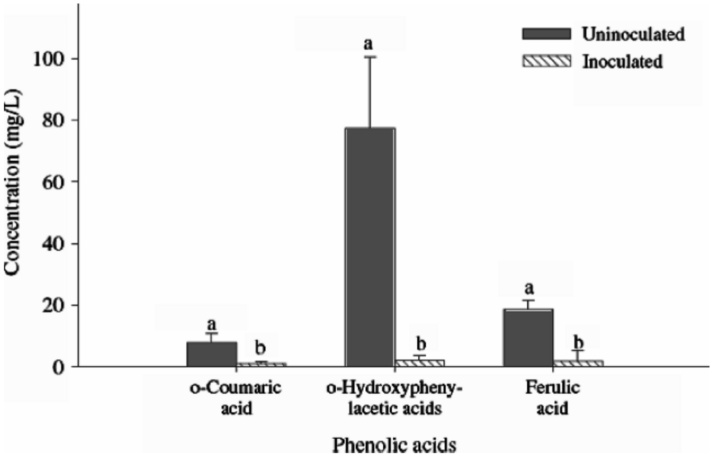

Fig. 5. Concentrations of o-coumaric, o-hydroxyphenylacetic, and ferulic acids in growing media containing uninoculated (control) and inoculated black spruce seedlings with *P. involutus* 211804 (reproduced from Zeng and Mallik, 2006)

Although some success of *Kalmia* control by commonly used herbicide glyphosate have been reported (Titus and English, 2000) a non-chemical option would be preferable. Public opinion in Ontario and Quebec Canada does not favor herbicideuse in forestry (Wagner et al., 1998). Since 80% of fresh water comes from forest streams concerns has been expressed because of potential the harmful effect of synthetic chemicals on fresh water and toxic effects on berry yielding plants such as bleuberry and red raspberry which are important food sources of wildlife. Blueberry picking after forest harvesting and wildfires is an important pass time and a source of income for many Canadians. Silvicultural herbicide treatment to control competing plants in young plantations can enhance conifer growth, but affect growth and yield of blueberry which in turn can affect wildlife that depend on berry plants (Moola, Mallik and Lautenschlager, 1998; Moola and Mallik, 1998). Since 2000 herbicide use in forestry has been banned in Quebec. It is essential to develop alternative methods of vegetation control in early stage of forest development when planted species often suffer from serious competition (Jobidon, 1991a). Several authors experimented with scarification and fertilization treatments in order to control *Kalmia* after forest harvesting and fire (Richardson, 1979; Thiffault, Titus, and Munson, 2004). It has been suggested that scarification followed by planning with larger seedlings and spot fertilization with nitrogen immediately after harvesting can be used to overcome *Kalmia* growth inhibition (Thiffault, Titus and Munson, 2005). These are encouraging results. However, success of black spruce growth in *Kalmia* dominated sites achieved by these experimental treatments has yet to be demonstrated operationally.

19.6 Concluding Remarks

With respect to forested ecosystems, the previously defined concept of allelopathy as a localized plant to plant interaction via release of allelochemicals has been expanded in recent years to an ecosystem-level phenomenon. In fire adapted ecosystems, forest fires reduce the amount of allelochemical by thermal decomposition as well as by adsorption in charcoal and create favorable post-fire seedbed condition. Conversely, the removal of forest canopy by fire and forest harvesting can stimulate vegetative regeneration of understory plants with high allelopathic and competitive properties. This allelopathic shrub dominated vegetation often resists forest regeneration, builds up allelochemical rich-humus bringing about retrogressive succession and habitat degradation. Recent studies in the boreal forests of Sweden have elucidated this complex interplay between forest fires and allelopathy at a landscape scale. Forest managers can take advantage of this new knowledge and develop and implement new management plans accordingly. It is indeed very encouraging that we are beginning to understand the complexity of landscape-level forest allelopathy with respect to ecosystem disturbance. However, it is also noticeable that the old habit of highly simplified experiments on allelopathy and inappropriate interpretation of data still exists.

Research on allelopathic weed control in forestry is inadequate albeit some encouraging results of past studies such as use of straw mulch to control competing shrubs in young plantations. Development of new herbicides from allelochemicals seemed challenging due to (i) their lower potency compared to the existing synthetic herbicides and (ii) absence of some desirable properties of the bioactive compounds. Nonetheless, herbicide such as balaphose developed from bacterial metabolites has been successfully used as a selective herbicide in forestry and agriculture. Other non-chemical approaches such as inoculation of tree seedlings with appropriate mycorrhizal fungi and scarification followed by spot fertilization at planting to overcome competitive and allelopathic growth inhibition in tree seedlings have good potential. What is needed at the moment is infusion of substantial funds to conduct creative experiments and field trials in order to develop new methods of weed control in forestry that are ecologically, economically and socially acceptable. New insights in rhizosphere ecology of allelopathic plants using advanced molecular techniques and interdisciplinary collaboration can help achieving these objectives.

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Index