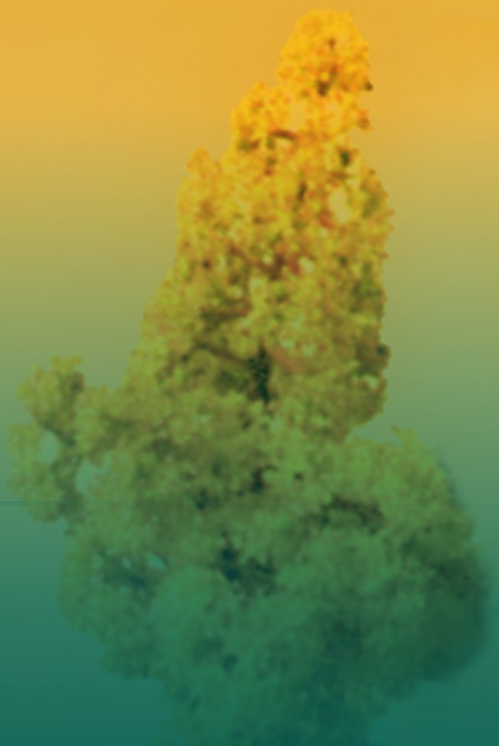
D. K. Chakrabarti



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Dedicated to my beloved parents Late Snehamoyee and Prasanta Kumar Chakrabarti

Preface

The economic impact of malformation disease of mango, one of the most important among fruit crops in the Indian sub-continent, is so serious that it fascinated the scientists of at least four different disciplines viz. plant physiology, horticulture, entomology and plant pathology (mycology and virology). But instead of combined multidisciplinary efforts to sort out the problem, the scientists in the Indian sub-continent and Egypt made piece meal approach, remained confined to the fragmented knowledge of their respective disciplines, refused to appreciate the merits of research in other disciplines and thus created confusion about the nature of the cause of the disease and failed to suggest field effective control measures. The scientific arguments were degraded into personal bickering to the extent that others who did not belong to any of the camps preferred to play safe and referred the disease as a "malady of unknown origin". Later the scientists of Israel, South Africa, USA, Mexico, Central America, Cuba, and Australia have participated in the research of mango malformation after appearance of this disease in their respective countries. The scientists of these countries are unequivocal about the nature of the causal organism. In India also the scenario has seen a gradual shift during the last twenty years. A consensus is being built up accepting *Fusarium moniliforme* var. *subglutinans* as the inducer of the malady. A single step control measure has been replaced by an integrated management strategy. However, the confusion that prevailed over several decades has not been totally resolved in the mind of some academics. The present monograph aims to address them with critical appraisal of the current status of the researches on this disease of international importance.

Faizabad, India D. K. Chakrabarti

Acknowledgement

In early seventies when I was a post-graduate student fortuitously came across for the first time a malformed plant in Varanasi, India, I was instantly attracted by its unusual appearance. Fortunately, my research supervisor Dr. S. Ghosal , Professor, Banaras Hindu University, indulged me to take it up as my Ph.D. research problem. My co-supervisor, Dr. C. Sen, Professor, B. C. Agriculture University, who had already been shot into fame for his research on mango black tip disease, prepared a strategy for me to deal with such a complex as well as socially sensitive plant disease. Time can not erode his interest in mango malformation and affection for me. Professor Sen inspite of his inclined health has painstakingly gone through the manuscript and corrected it with the same spirit as he used to in my college days. He has also kindly written the Epilogue of the monograph. Later after leaving the college, when I was struggling hard to make my passion of research on the malformation a profession, Dr. B. P. Singh, Deputy Director, National Botanical Research Institute, Lucknow provided me the much needed succor. A number of colleagues from different disciplines of plant sciences and students afterwards joined me, may be primarily due to affection for me or for obtaining their master degree. But soon they themselves like me became passionately involved with the problem. Without their unstinted help, many aspects of the disease could not be unveiled. To name a few are Dr. Kanika Biswas, Professor Banaras Hindu University, Dr. Vinod Singh, Principal Scientist, Centre of Sub-tropical Horticultural Research and Dr. Rajesh Kumar, Plant Protection Officer (U.P. Govt.).The research contributions of my other associates have been mentioned in the text.

I shall never forget the farmers at Rudauli-Sohawal mango belt of Uttar Pradesh who reposed unflinching faith on our research and allowed us to conduct experiments in their orchards, their only asset and extended ungrudging cooperation all through. Their plight due to this destructive disease and expectation for remedy from us, were the driving force for my team.

I thank the almighty as he has blessed me with a supporting family. No words can acknowledge the indebtedness to my sister, Dr. Rina Chakrabarti, Professor, Delhi University, my son engineer Pinaki Chakraborty, Computer Scientist, Jawaharlal University and my wife Rekha . But they vehemently opposed to spell out my emotion for them. So I acknowledge their contribution without speaking anything.

I am highly thankful to Zuzana Bernhart, the Senior Publishing Editor, Springer and her assistant Elisabete Machado. When at one time I got nervous wondering if the readers would accept the monograph, Zuzana's encouraging letters worked a magic on me.

Different professional societies like Indian National Science Academy, Indian Science Congress Association, Kothari Scientific Research, Uttar Pradesh Council of Agricultural Research, Indian Society of Mycological and Plant Pathology etc. encouraged me and my students by bestowing various prestigious awards. We are grateful to them.

I also acknowledge with gratitude the generous financial assistance in form of research projects from various agencies viz. Uttar Pradesh Council of Agricultural Research, World Bank, Indian National Science Academy, and Indian Council of Agricultural Research.

I am aware that we have 'miles to go' to completely unwind the mystery of malformation and find out easy and a single stroke solution. In view of the current enthusiasm among the new generation scientists and their improved techniques, I am hopeful that it is not far away when the stride to tame the devastating malady will trump the success of century old efforts.

Contents

Introduction

Mango is one of the world's most important fruit crops. Mango was originated in the Indo-Burma region from where it travelled to different parts of the world since the sixteenth century. Mainly the Muslim missionaries, the Spanish voyagers and the Portuguese introduced the mango from India to different countries (Fig. 1). Thus, besides India, mango is now being cultivated in about 85 countries. Important countries growing mangoes are China in far east, Philippines, Indonesia, Thailand, Burma, Malaysia and Sri Lanka in south-east Asia, Egypt, south-east Africa, South Africa, Israel, tropical Australia, the USA (Hawaii and Florida), Mexico, Brazil, Cuba and the islands of the West Indies. In 2004, world mango production was 26.5 million metric tons and total area under mango production was 3.69 million ha (www.natx.com). Top mango growing countries of the world and production scenario in 2007 are listed in the Table 1.

Almost each part of mango plant is used for different purposes. While wood is used as a timber, leaves and dried twigs are used for various religious purposes and the fruit is consumed raw or ripe. Raw fruits are used for making flour and drinks. Ripe fruits besides being consumed as dessert, are processed into jam, squash, slices, pulp, juice, nectar and mango leather. The kernel contains 8–10% fat which is used in soap industry. Its starch can be used in confectionary industry.

In India there are more than a thousand varieties of the mango which belong to one species, *Mangifera indica* L. The important commercial varieties are maintained in cultivation through vegetative propagation by grafting. They differ from one another mainly in fruit characters and a few other minor features like colouration of emerging leaves, colouration and pubescence over the panicle branches etc. In India, only three species of *Mangifera* have been reported which are (1) *M. indica* L., (2) *M. khasiana* Pierre and (3) *M. sylvatica* Roxb. In Malaysia, there occur 41 species of the genus *Mangifera* (Mukherjee 1950).

The genus *Mangifera* L. belongs to the family Anacardiaceae. The chromosome number of *M. indica* is 2n = 40 and n = 20 (Mukherjee 1950). On the basis of morphology, the chromosomes have been distinguished into 11 types, of which eight are distinct and three are intergrading (differences between the compliments are inconspicuous). The varieties of mangoes and allied species differ from one another mainly in assortments of these chromosome types. The primitive type(s) gave rise

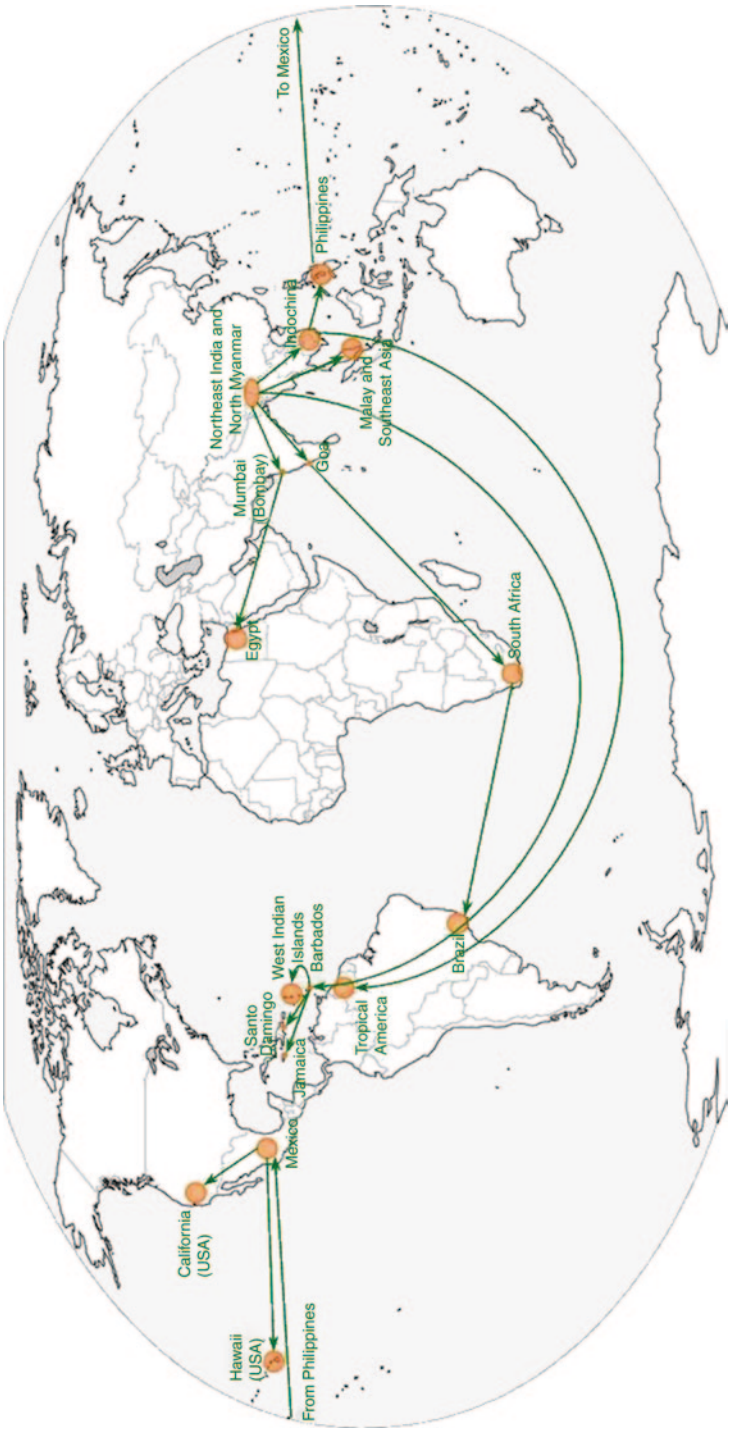

Fig. 1 The pathway of movement of mango cultivars from its place of origin to different countries

Table 1 Top mango growing countries in the world and production scenario in 2007. (Source: FAO, United Nation, Economics and Social Department. The Statistical Division)

Country	Production (million MT)
India	13.50
China	3.75
Pakistan	2.25
Mexico	2.05
Thailand	1.80
Indonesia	1.62
Philippines	0.98
Nigeria	0.93
Vietnam	0.37
World	33.40

to the mango varieties originated through alloplopolyploidy, most probably through amphidiploidy. The difference between numerous varieties took place primarily through gene mutations, the selected types being preserved under cultivation by grafting. The area of the maximum range of diversity is possibly the centre of origin of the species (Mukherjee 1950).

There are hundreds of varieties in mango, out of which only some are of commercial importance. The commercial varieties of mango, although having a wide range of adaptability, are specific to different sets of climatic factors. Thus, in India different regions have their own commercial varieties (Table 2).

Performance of the north Indian varieties undergoes marked change when grown under south Indian conditions and vice versa. For instance, if Langra and Dashehari of the north Indian varieties are grown under south Indian conditions the trees flower and fruit sparsely. Similarly, Neelum, a south Indian variety, tend to be sufficiently dwarf under north Indian conditions accompanied by reduction of fruit size and delayed ripening.

In India almost all the commercial cultivars are monoembryonic. A few that are polyembryonic (having more than one embryo in seed) are comparatively of little economic value and confined to the west coast of India. The seedlings arising from polyembryonic seeds are highly uniform and can be used as such for vegetative multiplication.

The present commercial varieties of mango in India by and large are alternate bearers. Neelum and Bangalora although are regular bearers but inferior in fruit quality. Therefore, for producing a regular-bearing variety with fruit quality acceptable to consumers researches were initiated at Indian Agricultural Research

Table 2 Commercially grown mango cultivars in different agro-climatic zones in India

Different regions in India	Varieties grown
Northern part	Bombay green (early), Langra, Dashehari and Chausa
Eastern part	Fazli, Kishenbhog, Himsagar, Langra, Gulabkhas and Zardalu
Western part	Alphanso, Pairi, Malkurad (Goa), Kesar, Rajapuri and Jamadar (Gujarat)
Southern part	Beneshan (Banganpalli), Neelum, Bangalora Rumani, Suvarnarekha, Mulgoa, Raspuri and Badami

Table 3 Important commercial mango varieties of different countries

Country	Varieties
India	Alphanso, Benishan, Kesar, Dashehari, Himsagar
China	Zipdieya, Mabrouka, Al-Fons, Kent, Kiet, Tommy Atkins
Pakistan	Sindhri, Anwar Rataul, Fajri, S.S.-1, Dashehari
Thailand	Brahman, Okrong
Indonesia	Golek
Mexico	Manila, Ataulfo, Haden, champagne, Kent, Kiet, Tommy Atkins
Sri Lanka	Ruby
Israel	Maya, Sheky
Australia	Kensington Pride, R2E2
South Africa	Heidi, Haden, Kent
Venezuela	Super Haden
Brazil	Extrema
Egypt	Hindi Besennara, Ewais, Genovea, Timour, Zebda
Vitenam	Xoai Tuong, Keow Savoey, Falam, Nam Klangwan
Florida	Haden, Kent, Kiet, Tommy Atkins
Philippines	Carabe
Kenya	Boribo, Apple, Ngowe

Institute, New Delhi (IARI). Among the hybrids developed at IARI, Mallika and Amrapali (a cross between Neelum and Dashehari) have already been very popular. Apart from above, in recent years many more hybrids have been released which are dwarf, regular bearers with attractive skin colour. However, international trade of mangoes is dominated by varieties like Keitt, Tommy Atkins, Alphanso etc. The important commercial mango varieties in different countries are listed in the Table 3.

Mango is very well adapted to tropical and subtropical climates. It thrives even at an altitude of 1,500 m. However, it cannot be grown commercially in areas above 600 m. It cannot stand severe frost, especially when the trees are young. Dry weather before flowering is conducive to profuse flowering. Rains during flowering is detrimental to the crop. Strong winds and cyclones during fruiting seasons can play havoc as they cause excessive fruit drop. Mango starts flowering early in eastern States of India viz. West Bengal, Bihar and eastern Uttar Pradesh due to onset of high temperature early in the season. In the south under moderate temperature conditions even during winter the flowering may start in September–November. In some coastal areas (e.g. in Kanyakumari in India) there are varieties that flower and fruit twice a year (off-season bearing). The off season bearing is conditioned by the differences in night and day temperatures and humidity.

The mango is a deep-rooted tree and requires soil profile of at least 2 m depth. It grows well on wide variety of soil except extremely sandy, rocky, waterlogged, heavy textured and alkaline and calcareous soil.

Although a number of propagation techniques have been suggested, inarching although cumbersome and time consuming, is the only technique in vogue. Veneer grafting has started gaining grounds in recent years for mass scale commercial propagation.

A large number of insect pests and diseases attack mango crop, causing damage to all parts of the plants. More than 492 species of insects, 17 species of mites and 26 species of nematodes are known to infest mango trees, about 45% of these have been reported from India. Almost a dozen of them have been found damaging the crop to a considerable extent causing severe losses, and therefore, may be termed as major pests of mango. These are hoppers, mealy bugs, inflorescence midge, fruit fly, scale insects, shoot borer, leaf webber and stone weevil. The insects other than these are less injurious to mango crop and are placed in the category of minor pests.

Mango suffers from several diseases at all stages of its life. All the parts of the plant, viz. trunk, branch, twig, leaf, petiole, flower and fruit are attacked by a number of pathogens including fungi, bacteria and algae. They cause rot, die-back, anthracnose, scab, necrosis, blotch, spots, mildew etc. Some of these diseases like powdery mildew are of great economic importance as they cause heavy losses in mango production.

In addition to diseases and insect pests, mango crop also suffers from many physiological disorders. Of which black tip of fruits, fruit drop, clustering in mango fruits and biennial bearing are very serious particularly in northern States of India.

The pests and diseases of mangoes have been generally well investigated and largely managed; suitable pesticides have been developed for all major biotic pest problems. Besides, all the pests and diseases may not be found in every mango growing countries and many are localized in a particular region with sporadic appearance in some years.

At present malformation has emerged as a serious threat to the mango industry the world over and has been designated as a plant disease of international importance. The disease has drawn wide attention from different quarters for the following reasons: (1) the disease infects the inflorescence converting them into malformed and unproductive bunches; thus, causing direct loss in yield and that too every year; (2) affects the growth and vigour of plants; (3) in nursery it produces bunchy top on seedlings and kills the root stocks; (4) due to the prevalence of the disease, there is a restriction on export of mango saplings from India; (5) the disease is wide spread, prevalent in all the mango growing countries in the world; (6) once the plants are infected, they remain diseased throughout i.e. the disease is endemic; (7) etiology and epidemiology are poorly understood; (8) all the commercial varieties and newly developed hybrids are apparently susceptible and (9) failure to find out satisfactory control measures. This uniqueness has proved to be an enigma, and has drawn the attention of scientists all over the world for decades. This monograph is a humble effort to present a critical appraisal of existing information on various aspects of mango malformation.

Chapter 1
Chronological History of Mango Malformation

In recent years no other plant disease has drawn so much attention from scientists of various disciplines and generated such high-pitched animated debate as mango malformation. The sequence of events that unraveled the confusion in understanding its cause and thereafter stepwise revelation of different aspects of the disease leading to a common agreement about the nature of its causal agent and developing integrate management practices makes a fascinating story. In this chapter attempts have been made to trace the course of research on mango malformation since its first report in 1891 till date. The publications that have mooted new ideas and directed the course of the investigations have been specially mentioned.

Maries, an expert mango grower in Darbhanga district of Bihar, first noticed mango malformation and Watt reported it in 1891 in the Dictionary of the Economic Products in India as the disease of mango panicles and stated that *irrigation carried on all the year round bring the malady*. It was later (1910) redescribed by Burns from Pune as malformation of mango inflorescence. Subsequently (1920) it was studied in some details by Burns and Prayag. Burns stated, *during the fruiting period, there is a phase of vegetative activity, commencing from the beginning of April; hence the inflorescences that are produced in April or later are partially influenced by this phase and the flowers are changed to leafy structures. It is not caused by any insect or fungus.*

In the initial years i.e. up to the fifties, the research was limited to the visual observations reporting the disease symptoms and severity; attempts were also made to speculate the probable cause and possible remedies. During this period eriophyid mites were the prime suspects as causal organism. Besides the hypothesis of virus origin of the disease was mooted, both vegetative and floral malformation were envisaged as the manifestations of the same disease and attempts were made to reduce the disease incidence through eradication of malformed plant parts.

Malformation in Uttar Pradesh In Uttar Pradesh, that has been generally the hot spot for mango malformation, the disease was noticed in 1933 by Singh and Chakravarti. They at Banaras Hindu University, Varanasi (BHU), observed "abnormal inflorescence" of mango on certain mango trees and within next two years

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about 22% plants in the campus of BHU were found to be severely affected causing an average 6–7% loss in yield (Singh and Chakravarti 1935).

Mite and Virus Hypotheses Next the disease was reported from Punjab (Singh et al. 1940). In 1944 Hassan and in 1946 Sayed in Egypt observed a species of Eriophyid mite, *Aceria mangiferae* Sayed was associated with malformation, both vegetative and floral shoots of mango, and perceived it to be the causal organism. But they did not carry out any test to confirm the pathogenicity. Speculation of virus origin of the disease which took long years to clear off was mooted in 1946 when Sattar from Punjab Agricultural College and Research Institute, Lyallpur reported that *It is not caused by any insect pest. Furthermore no fungal organism has been traced to be the cause of the disease. ...it is indicated that the disease may be of virus origin. It is also possible that the disease may be due to some physiological disorder. ... in light of the experience gained on other diseases it is recommended the malformed inflorescence should be removed from the trees and burnt. The operation is very likely to reduce infection.*

Vegetative Malformation Vegetative malformation was noticed for the first time in 1951 by Garg on grown-up trees in Uttar Pradesh. He described both floral and vegetative malformation as bunchy top. He speculated that the disease might be caused by a virus. Vegetative malformation on seedlings was recorded by Nirvan (1953) from Saharanpur, Uttar Pradesh. He restricted the term "bunchy top" to the vegetative malformation on young seedlings only. The idea that both vegetative and floral malformation may be two separate manifestations of the same disease was first conceived by Tripathi (1954) when he observed high correlation in incidence and severity between bunchy top and floral malformation and he termed it mango malformation. However, the experimental proof of this hypothesis was made available almost after two decades. So far all the reports were based on field observations. Tripathi (1955) initiated planned experiments. He treated malformed plants with different macro- and micronutrients to confirm whether the reduction in growth of the diseased plants was due to deficiency of any of the nutrients. When he did not find any consistent positive response with macro- and micronutrient treatments, he concluded that the disease was not caused by any nutrient deficiency. The time-tested and widely accepted control measure was proposed again in 1959 when Narasimhan confirmed that systematic removal of the diseased inflorescence(s) resulted in the disappearance of the disease.

The researches in the sixties are marked by systematic approach to identify the cause of the disease. Attempts were made to prove Koch's postulates for the first time both with eriophyid mites and a fungus *Fusarium moniliforme,* the two major suspected causal organisms.

Carbohydrates and Phenols The substantial increment in contents of carbohydrate and phenolic compounds drew the attention of the scientists and the interest was sustained for a long period. Hamid (1960) estimated higher accumulation of starch and tannins in malformed inflorescences and found foliar spraying of urea at monthly intervals during winter reduced malformation. Khan and Khan (1963)

recorded higher amount of carbohydrates in malformed panicles and suggested that imbalance of C/N ratio in malformed panicles was responsible for its disturbed sex ratio.

Koch's Postulates In 1961, Singh et al. reproduced malformed panicles while Puttarudriah and Channa-Basavanna (1961) reproduced malformed shoots by inoculating just sprouting floral leaf buds with mites taken from malformed twigs. They also attempted to confirm the virus origin of the disease. However, the result was negative as they observed that the disease was not graft transmissible. However, they identified a late flowering, monoembryonic variety Bhaddauran as resistant. In 1966, Summanwar et al. reported association of *Fusarium moniliforme* Sheld. with malformed flower buds and reproduced malformed shoots by artificial inoculation of isolated fungus. He (Summanwar 1967) observed the presence of fungal spores over body surface of the mites and presented experiential evidence that mites act as the vector of malformation. This was later confirmed by many scientists in different countries.

Temperature Effect The role of temperature on the disease manifestation which has become a favourite aspect of investigation in later years, was initiated in 1963 when Jawanda observed correlation between earliness and susceptibility to malformation. Singh et al. (1965) suggested that temperature at the time of panicle development have a great bearing on the production of perfect flowering. Higher maximum and minimum temperature during this period seem to favour higher number of perfect flowers. Based on these observations later a number of control measures were recommended.

The research in seventies witnessed an intensive investigation on the biochemical and physiological alteration in malformed plants. The horticulturists and plant physiologist interpreted the changes as the cause of the malady while the plant pathologists viewed them as the resultant of the pathogenic invasion (*F. moniliforme*) or physiology of pathogenesis.

Deblossoming and NAA Spray During this time a control measure consisted of deblossoming and naphthyl acetic acid (NAA) spray which is till date the favourite recommendation for horticulturists and plant physiologists was suggested. The rationale and gradual realization of this control strategy are very interesting. In the late sixties, Jagirdar and Jafri (1966) for the first time related the disorder to the imbalance of auxin and anti-auxins. Majumder et al. (1970) endorsed the above hypothesis and assumed that number of hermaphrodite flowers in malformed panicles might be due to depletion of auxin. They applied NAA (200 ppm) at the first week of October and got reduction in malformation. The other line of thinking was that the reduction of fruit setting was due to lack of pollination. Singh et al. (1974) presumed that non-availability of pollens for the early flush of panicles in cv. Dashehari which is self-incompatible may be responsible for less fruit setting. Thus they suggested that single deblossoming at bud burst stage increases productivity. Majumder and Sinha (1972) identified low temperature at the time of flowering as a contributing factor towards development of mango malformation. Based on the

above observations, Majumder et al. (1976) recommend treatment of the malformed plants with NAA (200 ppm) in late December or early January when panicles were just emerging followed by deblossoming. The primary purpose of deblossoming was to shift the emergence of the early panicles to a later date when the temperature became relatively higher (Jawanda 1963). Besides the warm weather was more conducive to efficient pollination by pollinating insects.

Plant Proteins Sandhu (1975) reported inhibition of protein synthesis in malformed shoots that resulted in greater number and amount of free amino acids but lesser amount of bound amino acids. He interpreted that protein deficiency caused reduction in leaf size. Excess bound amino acids accompanied by less free amino acids led to the formation of large number of tiny branches and inhibition of apical dominance. Abou-Hussein et al. (1975) recorded high level of gibberellins in malformed florets and they linked it with abnormal expression of sex in malformed inflorescences. Pandey et al. (1975) observed reduction in DNA and RNA contents in malformed panicles.

Changes in Perspectives During 1970s The horticulturists and plant physiologists estimated the biochemical constituents of the malformed plants and attributed the aberrant biochemical constituents as the cause of the malady. On the contrary, plant pathologists first inoculated the healthy plants with the pathogen and subsequently reproduced the similar biochemical changes. Thus convinced that the abnormal biochemical constituents were not the cause of the disease; on the contrary, these were the results of the pathogenic invasion.

Chattopadhyay and Nandi (1977b) after inoculating a healthy plant with the pathogen recorded rapid increase in protein nitrogen and soluble nitrogen. Similarly, there was gradual increase in total phosphate content (Chattopadhyay and Nandi 1977b) and marked degradation of celluloses and lignin (Chattopadhyay and Nandi 1977d) with advancement of the disease. After infection they recorded increased activity of peroxidase and polyphenoloxidase (Chattopadhyay and Nandi 1976) and related it with the disease resistance ability of the host. Incidentally, it was the first report of enzyme activity of the malformed plants.

Pathogenic Nature of the Pathogen The pathologists (Varma et al. 1974) during this period renamed *F. moniliforme* as *F. moniliforme* Sheld. var. *subglutinans* Wollenewb and Reinking and reproduced floral malformation by inoculating with the fusarial strain isolated from malformed vegetative shoots and vice-versa and thus proved experimentally the common etiology of the two malformations. They also recorded that the fungal growth was restricted to certain cells and did not spread in the host cells systemically. Ibrahim et al. (1975) reported that only certain isolates of *F. moniliforme* could induce the disease and wound(s) were essential for entrance of the pathogen into the host. This was the primary indication towards host specificity of the pathogen, failure of the pathogen to produce cell wall degrading enzymes in host cells and its dependence on external agencies like mites to enter the host.

Micronutrient Deficiency: The Function of the Pathogenic Invasion The earlier proposed imbalanced C/N ratio hypothesis was interpreted differently by Pandey

et al. (1973). They observed that like healthy shoots, malformed shoots also contained high C/N ratio but failed to develop normal flowers. He suggested that it might be due to blocking up of translocation of metabolites from leaves to the developing buds in malformed shoots by some toxic metabolites of a pathogen. More information in this direction was generated later.

The Fusarial Toxins and the Host Metabolite in Symptom Production In 1979 (Ghosal et al.), the presence of toxins of *F. moniliforme* var. *subglutinans* was detected in the malformed cells; the chemical nature of the toxins and their role in producing the disease symptoms were established. The phenol content in the malformed cells as reported earlier by Hamid (1960) and Prasad et al. (1965) was identified as mangiferin and its role in malformation was delineated. Mangiferin in high quantity inhibited the pathogen inside the host but due to its attendant side effects there were several biochemical and physiological imbalances which in turn were expressed as the disease symptoms. Commonly in fusarium-induced plant diseases, the fungal toxins and enzymes are reported exclusively to produce the disease symptoms. But in mango malformation in addition to the fusarial toxins the aberrant host metabolites also played apparently a significant role in the disease manifestation. This aspect was not been taken into consideration by the earlier plant pathologists. But this is a point where the horticulturists and plant physiologists were partially correct when they observed that the aberrant host metabolites are linked with the disease symptoms.

The spread of the disease to distant places with planting materials was brought into focus by Malo and McMillan (1972) which underlined the necessity of implementing quarantine measures. How the pathogen has spread internationally was revealed by the studies on the population genetics with the pathogen in 2002 by Zheng and Ploetz. El-Ghandour et al. (1979) estimated micronutrients in malformed leaves and observed zinc, copper, manganese and boron were at lower level over healthy tissues. This was first direct experimental evidence of micronutrient status in malformed cells.

In the eighties, most of the research publications directly or indirectly substantiated the pathogenic origin of the disease. In addition to the estimation of growth hormones in malformed tissues, interest in the disease epidemiology was also become apparent.

Role of the Fungal Toxins Role of the fungal toxins in malformation was reported subsequently. Kumar et al. (1980) reported the pathogen to produce two strong inhibitors both in culture filtrates and in malformed tissues. In 1984, Ram and Bist proposed the malformin hypothesis. They reported the presence of a malformin-like substance in malformed tissues and application of malformin caused malformation in *Phaseolus vulgaris.* Malformin is a phytotoxic compound produced usually by *Aspergillus niger.* Later they (Kumar and Ram 1999) presumed that malformin might be produced in the malformed tissues by the fusarium pathogen. Kishtah et al. (1985b) examined malformed tissues through electron microscopy; they also made forays into transmission, cultural and serological tests that did not reveal the presence of any virus in malformed tissues. This ended all speculations of the

virus origin of the disease. Bist and Ram (1986) observed the pattern of changes in gibberellin(s) content of developing malformed panicles. It was different from those in healthy panicles. They concluded that the difference in pattern was due to the synthesis of gibberellins by *F. moniliforme.* The malformed tissues not only contained higher amount of cytokinins but it differed qualitatively from that present in healthy materials (Bist and Ram 1986; Van Staden et al. 1989). The cytokinins produced by the fungus in cultures were detected in malformed flowers but not in healthy flowers (Van Staden et al. 1989). The differences in phenolic and steroidal content(s) between malformed and healthy florets were reported and the importance of these compounds in the disease manifestations was realized (Ghosal and Chakrabarti 1988).

The earlier reports that deficiency of nutrients in the malformed plant parts was due to disruption of transport system in such plants were further investigated. Ibrahim and Foad (1981) studied the histopathology of the malformed inflorescence. The xylem vessels of the malformed leaves were poorly developed. A disease cycle was proposed in and the pathogen-induced biochemical changes were linked with the disease symptoms. Earlier studies (Pandey et al. 1973; Ibrahim and Foad 1981) suggested that lack of transportation of micronutrients from leaves to the growing points partially affected the normal development of the shoots and inflorescence. Chakrabarti and Ghosal (1989) who identified mangiferin as the carrier molecule(s) of metal ions in mango, further elaborated on the above observations. Due to immobilization of mangiferin at the infection site, the transport system in malformed plants was disrupted. Thus removal of malformed plant parts followed by spraying with mangiferin metal chelates elicited substantial recovery the plants. Shawky et al. (1980) reported that more than 60% of the malformed panicles were born on shoots of the previous year's March-April followed in decreasing order by those borne in June–July and September shoots.

Despite the strong evidence supporting *F. moniliforme* var. *subglutinans* as the causal organism of the disease, some publications cast doubt on the fact that the fungus is responsible for mango malformation. Hence, to provide unequivocal evidence, a series of studies using molecular diagnostic tools were undertaken in the nineties. This period also evidenced large number of publications on the epidemiology that was so far poorly understood. Besides some disease forecasting models were also proposed.

Host Specificity Kumar and Chakrabarti (1992) produced biochemical evidence to confirm that a strain of *F. moniliforme* isolated from malformed mango tissues can only induce the disease and thus it was a physiologically specialized strain. Leslie (1995) studied the mating behavior of species of *Gibberella fujikuroi* and on the basis of the data on vegetative compatibility (VCG) study, he suggested that strain of *G. fujikuroi* has adopted specifically to mango. Freeman et al. (1999) transformed a wild strain of *F. moniliforme* var. *subglutinans* with GUS reporter gene. When the transformants were inoculated into healthy mango buds, typical malformed shoots/panicles developed. The presence of GUS—stained mycelium of the pathogen in infected tissues proved that *F. moniliforme* var. *subglutinans* indeed was the causal

agent. Ploetz (1994) observed the presence of the fungus even on non-malformed shoots/panicles although in small number. This raised doubt about the pathogenic ability of the fungus. However, he explained that threshold level of infection may be required before development of the symptoms. Kumar and Chakrabarti (1995) presented experimental evidence to show that isolates of *F. moniliforme* undergo certain biochemical changes during prolonged association with *Mangifera indica* and only after this transformation, the wild strains of *F. moniliforme* can enter the mango.

Epidemiology The spatial pattern of spread of floral malformation in regular and alternate bearing cultivars and structure of the epidemic were reported by Kumar and Chakrabarti (1997b). The disease dissemination from plant to plant is very slow but a small amount of inoculum can initiate the epidemic within a couple of years. These observations were later utilized in formulating IPM strategy. Subsequently seasonal variation in *F. moniliforme* population in relation to environmental factors, mangiferin content and flushing in mango was investigated (Chakrabarti et al. 1997). Babu-Koti and Rao (1998) reported the biochemical changes and dynamics of the growth of the pathogen in the host cells during different months of the year. The effect of temperature on the disease development was also studied under controlled conditions (Singh et al. 1998). A new group of mite (mycophagus) was reported to act as the vector of the pathogen. The mite showed its dependence on the fungus as the mite used the fungus for its feed. Thus, for the first time interdependence between the *Fusarium* and the mite was revealed (Chakrabarti et al. 1997a). Maksoud and Haggag (1995) proposed a model for forecasting percentage of floral malformation using quantity of micronutrients or plant growth regulators in host cells as predictors. Another predicting equation was also published in 1997a (Kumar and Chakrabarti) to forecast quantum of loss in yield in different cultivars of mango using number of malformed panicles as predictors. These were followed by publication of a few more predicting equations in later years.

Initially the disease was either absent or sporadic in the coastal states of India. It was presumed that constant high temperature may not allow the disease to thrive there. But later it was observed that the disease after introduction into West Bengal, a coastal state, with planting materials not only has established well but also being spread among local cultivars. But the disease manifestation under uncongenial conditions showed variations and the pathogen seemed to lose its virulence to some extent (Chakrabarti and Kumar 1997, 1999).

Epidemiology Based Control Strategy In the beginning control measures were symptom—based. Thereafter, control measures were recommended as per perceived nature of the cause of the disease—either biotic (mite and fungus) or abiotic (hormones and nutrients). But since this period integrated management strategy gradually replaced the silver bullet application of a single pesticide or growth hormone became the norm. IPM strategies were formulated keeping the disease epidemiology in view (Noriega-Cantu et al. 1999; Kumar and Chakrabarti 1998).

In the past few years, also a plethora of convincing experimental evidence supporting *F. moniliforme* var. *subglutinans* as the causal organism have been generated. Its taxonomic position was reviewed and nomenclature of *F. moniliforme* var. *subglutinans* from mango was revised. Subsequently many new aspects of the epidemiology have been focused.

Molecular Tools to Confirm the Identity of the Pathogen Earlier on several occasions (Singh et al. 1961; Prasad et al. 1965; Salama et al. 1979) attempts to reproduce the disease symptoms by artificial inoculation failed which raised doubt about the pathogenicity of the fungus. Chand and Chakrabarti (2003) identified PR proteins in mango that prevented the pathogen to colonize and invade. They inhibited the translation process that resulted into synthesis of PR protein which concomitantly helped the pathogen to colonize. The improved inoculation techniques has made it possible to reproduce both floral (Chand and Chakrabarti 2003) and vegetative malformation (Chand and Chakrabarti 2004; Misra and Singh 2005) consistently and easily.

Britz et al. (2002), Zheng and Ploetz (2002) and Marasas et al. (2006) using different molecular diagnostic tools such as nuclear and mitochondrial DNA sequences of several genes and isozymes and tests for mating type and sexual compatibility concluded that the *Fusarium* isolate from malformed mango tissues represented a new species in *G. fujikuroi* complex and is a discrete taxon. They described it as a new species, *F. mangifera* Britz., Wingfield and Marasas *sp. nov.* The chromosomal anomalies during microsporogenesis in malformed flowers resulting into formation of abortive pollens was reported in 2001 (Kumar and Chakrabarti).

The studies on population genetics conducted with the *F. mangifera* (Zheng and Ploetz 2002) showed very little variations amongst isolates from Florida, Egypt, India, Israel, Malaysia and South Africa. The low number of VCGs indicates that populations of this pathogen reproduce clonally. *F. mangifera* from different geographical areas was most probably introduced from India. It was assumed that the pathogen have originated in India.

The conidia of *F. moniliforme* var. *subglutinans* serve as the infectious entities. Therefore, to understand the infection process various aspects concerning these infectious units such as time taken for conidia production, number of conidia produced in different season, effect of temperature and moisture on conidia germination, effect of concentration of conidia on its germination and viability of conidia during different periods of the day were investigated in details (Pandey et al. 2005).

IPM For development of meaningfult management strategies the disease management attempts were made to understand the natural defense mechanism of the host and to include it in the IPM strategy. To find out how mangoes survive the epidemic in nature, it was observed that the disease incidence after continuous increase for 4–5 years (epidemic stage) attained a state of balance in host-patho system (endemicity) (Chakrabarti et al. 2005). The endemic stage was effected due to availability of smaller/weaker inoculum potential (colony forming units). During the endemic stage the defense system of the host was rejuvenated. Taking cue from the defense system of the host in nature, an integrated management strategy (IPM)

was developed with the objective to reduce the inoculum potential with the help of mangiferin copper chelates, pruning of malformed shoots and panicles and a natural antagonist, *Aspergillus niger* of *F. moniliforme* var. *subglutinans.* Pruning reduced disease incidence in the immediate next flowering/fruiting season. But if it is not repeated next year the rate of disease increment became very high (Pandey and Chakrabarti 2004). To protect mango plants from large number of biotic and abiotic stress, various pesticides, growth hormones and micronutrients are sprayed every year. Some of the commonly and intensively used chemicals were found to weaken the defense system of the host while some compounds stimulated the same (Kumar and Chakrabarti 2007). These observations further emphasize the importance of practicing an IPM strategy instead of tackling each of the disease or insect pests separately.

The Disease Forecasting The results of the experiments on identifying the malformation predicting parameters it was recorded that host age could be used to predict vegetative as well as floral malformation (Pandey et al. 2003). In a separate investigation the number of vegetative malformed shoots before flower bud initiation was successfully used to predict the incidence of floral malformation in the forthcoming crop season (Chakrabarti et al. 2003). Finally, a computerized expert system has been developed to predict the disease incidence in any State of India and to suggest appropriate IPM strategy (Chakrabarti and Chakraborty 2007).

Varietal Screening From the very beginning, a number of reports published included the performance of various cultivars of mango against the malformation under natural conditions. But recently emphasis has been given to confirm the disease resistance capacity of mango cultivars by artificial inoculation test and thereafter to be included in resistant breeding programme (Mishra 2004). The biochemical parameters particularly that of polyphenol content of the cultivar have also been suggested to consider in selecting the resistant cultivars (Wada et al. 2001; Singh 2006).

Epilogue Serious efforts are on way to develop malformation resistant plants mainly through genetic manipulation (Zaccaro et al. 2006). Although the disease resistant cultivars have been identified (Kumar et al. 1993), transfer of the resistant genes into commercial cultivars has not yet been possible due to lack of protocol for tissue culture of mango. With available technology, the disease can be managed satisfactorily. However, huge size of mango trees hinders undertaking of IPM measures that are to be applied at three to four stages. Thus, hanging malformed panicles on mango trees throughout the year has been a common sight in most of the mango growing areas. Strenuous efforts of hundreds of scientists over more than a century have culminated in unveiling the cause of the disease and also to manage it. But it is an irony that still some observers (as pointed out by Ploetz and Prakash 1997) try to keep the enigma of mango malformation alive and prefer a nebulous identity without assigning any reasons to refer it as a disease of unknown origin!

Chapter 2
Geographical Distribution of Mango Malformation

World Scenario Mango probably originated in the Indo-Burma region (Mukherjee 1953). Similarly, the pathogen of mango malformation, a physiological strain of *Fusarium moniliforme* Sheldon. var. *subglutinans* Wollenewb and Reinking (*F. mangiferae* Britz, Wingfield and Marasas *sp. nov.*) (Britz et al. 2002) has been assumed to have originated in India (Zheng and Ploetz 2002). The disease was first reported from India by Watt (1891). Mango has been under cultivation in the Indian Sub-continent for more than 4,000 years. From here it was taken to other Asian regions, Africa and America. The movement of grafted mangoes apparently started after 1860 (Purseglove 1968) and that must have been how mango malformation started spreading through inadvertent propagation of diseased plants. This is substantiated by the occurrence of the disease on Indian cultivars in Israel (Malo and McMillan 1972). The vegetative compatibility tests and the PCR assay of random amplified polymorphic DNA suggest that the isolates of the *Fusarium* pathogen from Florida, India, Israel and South Africa are closely related (Steenkamp et al. 2000). Britz et al. (2002) observed close uniformity in sequences of Histone *H3* and *β*-tubulin genes of isolates of *F. mangiferae* from South Africa, USA, Israel, Egypt and Malayasia and grouped these isolates into 'Asian Clade'. They viewed that *F. mangiferae* of different geographical areas was most probably introduced from India and *F. mangifeare* has introduced into areas such as South Africa and Israel as single genets (Marasas et al. 2006). The disease has already registered its presence in the continents like Asia (India, Pakistan, Bangladesh, Israel, Malaysia, and United Arab Emirates), Africa (South Africa, Egypt, Sudan, Uganda, and Swaziland), America (Brazil, El-Salvador, Mexico, Nicaragua, USA, Venezuela, and Cuba) and Australia (Table 2.1). In Egypt it was first noticed during 1934 and it became severe by 1958. Similarly, in Mexico the disease was first recorded in 1958 in many plantations of the States of Morelos, Guerrero and Veracruz. But by 1999, its occurrence was recorded from most of the states where mangoes are grown (Noriega-Cantu et al. 1999). In America, the disease was first noticed in southern Florida in 1969 from where it was distributed during 1970–1972 throughout Central America with infected planting materials (Malo and McMillan 1972). In Florida, malformation affected

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Table 2.1 The countries where mango malformation is prevalent

Year of first report	Country	Authors
1891	India	Watt
1944	Egypt	Hassan
1960	Pakistan	Khan and Khan
1961	Mexico	Morales and Rodriguez
1968	South Africa	Schwartz
1970	Brazil	Flechtmann et al.
1971	Sudan	Minessy et al.
1972	USA	Malo and McMillan
1972	El Salvador	Malo and McMillan
1972	Nicaragua	Malo and McMillan
1972	Venezuela	Malo and McMillan
1983	Cuba	Padron
1985	Malayasia	Lim and Khoo
1985	Swaziland	Crookes and Rijkenberg
1986	Australia	Peterson
1991	United Arab Emirates	Burhan
1992	Bangladesh	Meah and Khan
2008	Sultanate of Oman	Kvas et al.
2008	Spain	Kvas et al.

orchards were widely separated from each other confirming the spread of the disease through planting material. In United Arab Emirates also malformation was noticed for the first time on mango cultivars of Indian origin in 1989 in Dahid and Aweer areas. Schlosser (1971) did not find malformation in Bangladesh although mangoes are grown there extensively. The disease was noticed there in 1992.

Indian Scenario A survey of orchards of 18 districts of Western and eastern Uttar Pradesh (U.P.) was carried out. The survey revealed the disease was present all over U.P. In general the intensity of the disease was higher in western districts than in eastern (Prasad et al. 1965). Varma et al. (1974) found maximum incidence in the north-west region of India where nearly 50% of the plants were affected whereas in the north-east and south the incidence was less than 10%. Singh and Jawanda (1961) observed greater incidence of malformation in sub-mountain districts of the Punjab than in drier areas of the plains. Sharma and Badiyala (1990) surveyed the disease incidence at four different heights (500–850 m) above sea level of Himachal Pradesh in India. The disease incidence gradually decreased with increase in elevation. The disease has also been reported to make an appearance in Madras city, parts of Salem and Coimbatore (Prakash and Srivastava 1987). In Jammu or Himachal Pradesh the malformation was noticed on north Indian varieties like Chausa, Dashehari and Langra. Similarly in Malda mango belt of West Bengal, the outbreak of the disease was observed over the north Indian mango hybrids, Amrapali and Mallika

Table 2.2 The Indian states from where the disease has been reported

Year of first report	State	Author
1891	Bihar (Darbhanga)	Watt
1910	Maharashtra (Bombay)	Burns
1935	Uttar Pradesh (Varanasi)	Singh and Chakravarty
1940	Punjab	Singh et al.
1953	Uttar Pradesh (Saharanpur)	Nirvan
1961	Karnataka (Bangalore)	Puttarudriah and Channa-Basavana
1962	Gujarat	Desai et al.
1962	Delhi	Nariani and Seth
1973	Haryana (Hissar)	Khurana and Gupta
1975	Madhya Pradesh (Jabalpur)	Sharma and Tiwari
1975 (1973)	Jammu	Puttoo et al.
1975 (1972)	West Bengal (Burdwan)	Chattopadhyay and Nandi
1977	Uttarakhand (Pantnagar)	Bhatnagar and Beniwal
1979	Andhra Pradesh (Sangareddy)	Kulkarni
1989 (1985)	Orissa (Bhubanesar)	Das et al.
1990	Himachal Pradesh (Kangra, Solan, Hamirpur, Una, Bilaspur)	Sharma and Badiyala

The data in the parenthesis denote the year for first time of observation

(Chakrabarti and Kumar 1997). Singh and Jawanda (1961) recorded appearance of the disease on varieties (Bombay Green) in States of south India introduced from Bombay. The Indian States where the disease has been located are listed in the Table 2.2.

Chapter 3
Economic Importance

Malformation on young seedlings in the form of "bunchy top" at nursery stage causes loss to the nurserymen by reducing root stocks raised for propagation purposes. Singh et al. (1961) in Saharanpur (western U.P.) recorded 0.8% incidence out of 5,000 plants on 5 month-old seedlings which subsequently increased to 18.4% in next 4 months. Kumar and Beniwal (1992) reported about 10% incidence from Pant Nagar of UttaraKhand. Recently Gaur and Chakrabarti (2009) observed 0.7–4.25% seedling malformation and 0.5–67.3% malformation on one year-old grafted plants in nurseries of eastern districts of Uttar Pradesh. Often the malformed saplings with mild symptoms escape notice and are procured by farmers due to ignorance of the problem in establishing new orchards. But the infected plants never recover and thus farmers lose their entire investment. Moreover, the inadvertent propagation and distribution of malformed planting materials (malformed grafted plants as well as infected scion shoot) resulted in wide dissemination of the disease.

The malformation of panicles has profound bearing on the economy of the crop. If a large proportion of the fruiting spurs remain unproductive there is a significant loss to the orchardists. Tree losses up to 86% in one grove have been recorded over a three-year period (Kumar et al. 1993). The disease also inflicts indirect losses by reducing number of total panicles (Kumar and Beniwal 1992; Kumar and Chakrabarti 1997a). Upto Ca. 50% level of the disease severity, plants yielded more panicles to compensate loss of panicles rendered malformed; but with further increase in severity, flowering declined severely (Kumar and Chakrabarti 1997) (Fig. 3.1). Kumar and Beniwal (1992) reported shedding of fruits in healthy inflorescence of trees with a higher disease severity before maturity. But Kumar and Chakrabarti (1997a) did not find the increase in number of malformed panicles to cause shedding of florets from the panicles; rather they were indirectly related. However, later a linear regression equation was developed with which the extent of loss in yield could be predicted by counting the number of malformed panicles (Kumar and Chakrabarti 1997). They also recorded that in the cultivars viz. Langra, Himsagar, Gilas and Neelam the yield declined by 0.2, 0.6, 0.89 and 0.96% respectively for each 1% increase in malformed panicles. Incidence of floral malformation in on year as compared with that in off year was considerably higher (Kumar and Chakrabarti 1997).

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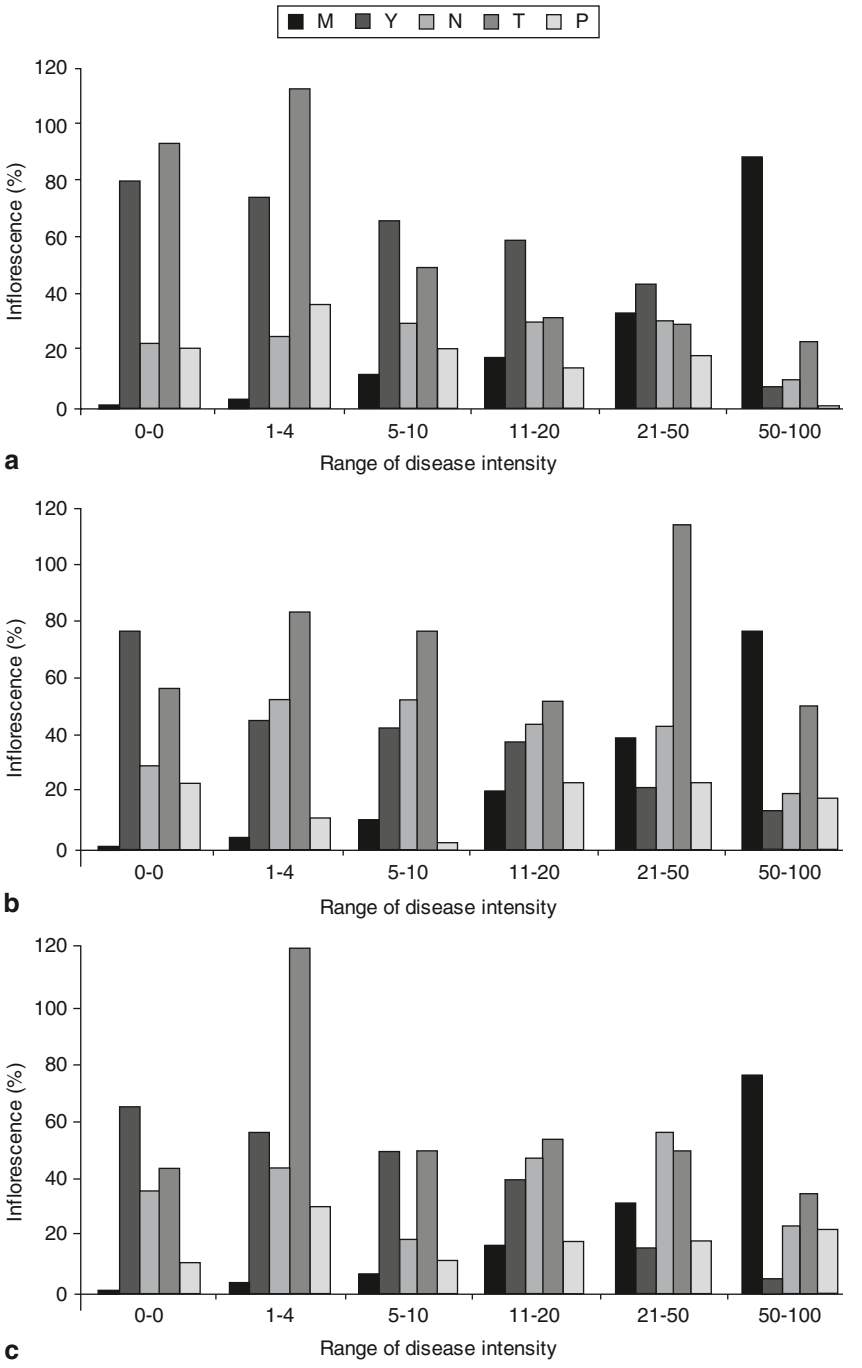

Fig. 3.1 Correlation between the disease intensity and yield in mango cvs. Dashehari (**a**), Langra (**b**) and Chausa (**c**)

Chapter 4
Symptoms

External Symptoms

Malformations in mango are of two types: (1) vegetative and (2) floral.

Vegetative Malformation

The symptoms of vegetative malformation have been recorded in detail by earlier workers (Nirvan 1953; Singh et al. 1961; Varma et al. 1974) and comparatively recently by Kumar and Beniwal (1992). A consolidated and comprehensive account of the symptoms described by them are presented here.

The vegetative malformation occurs on the young seedlings in the nursery as well as on grown-up trees of both grafted and seedling varieties. To distinguish the malformation on seedlings in the nursery stage from malformation on grown-up trees, it has been termed as 'bunchy top' (Fig. 4.1).

Seedlings

The disease starts with formation of a small shootlet from a swollen bud which bears small scale-like leafy structures at short internodes. The growth of this shootlet gets arrested and subsequently several similar shootlets again arise from the axil of scaly leaves. This process continues and collectively, a number of such structures give rise to the malformed bunch. The shootlets of the bunch are much thicker than the main stem of the seedlings. If the seedlings are attacked apically (Fig. 4.1a) at an early stage, they remain stunted and eventually dry up (Fig. 4.1c). There is a good number of mortality at the seedling stage. If plants escape mortality, the growth is very much retarded. But when the attack is at a latter stage, they may continue to produce healthy as well as malformed growth (Fig. 4.1b).

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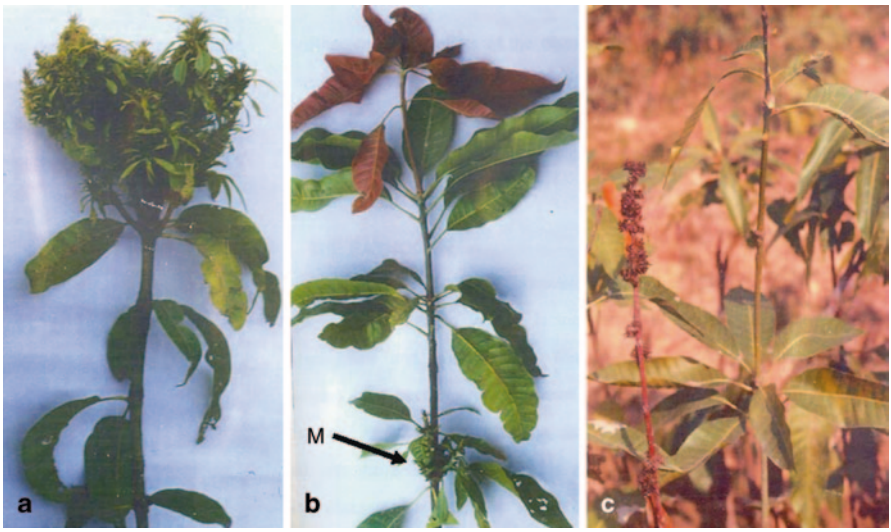

Fig. 4.1 Seedling malformation: bunchy top (**a**), malformation lower down the main shoot (**b**), and seedling mortality (**c**); (M = malformed shootlets)

Grown-up Trees

Vegetative shoots on grown-up trees (Fig. 4.2a) also get malformed by the development of whorls of small leaves on thick, stunted shootlets which crowd the apical portion or they may be formed in the axil of lower leaves. These malformed bunches dry up after a few months but the branches or twigs bearing them continue to grow inspite of the disease. Such compact branches dry up sometimes and remain attached to the shoots as dry masses (Fig. 4.2a). In certain case, these dry masses again produce malformed growth during the next growing season (Fig. 4.2a).

The axillary buds of dwarf branches are unusually enlarged. These buds persist even when dried and due to their crowding at nodes, form 'girdles' around branches. Such buds are referred to as 'scars' (Fig. 4.2b). Sometimes thick shootlets arise from swollen axillary buds developing into secondary branches that elongate further and bear small rudimentary leaves at the internodes (Fig. 4.2c).

Thus, the salient features of vegetative malformation are:
- Disturbance in apical dominance
- Excessive vegetative growth
- Branches swollen with short internodes
- Rudimentary leaves

Floral Malformation

Symptoms of floral malformation have been described by several scientists (Singh and Chakravarti 1935; Narasimhan 1954; Singh et al. 1961; Summanwar 1967;

Fig. 4.2 Vegetative malformation on grown up trees (**a**), '*scars*' on shoots (**b**) and thick malformed shootlets from scars (**c**)

and Kumar and Beniwal 1992). The symptoms recorded by them are summed up as follows.

Inflorescence

Singh and Dhillon (1990b) recorded morphological changes of developing floral organs of mango from its very inception. Panicle-forming buds at fully swollen stage and then flower buds at inception stage destined to develop into malformed panicles as compared with the normal ones were larger in both length and breadth.

However, later the normal panicles exceeded the malformed ones in length significantly. However, malformed panicles possess significantly higher spreads than the normal panicles. The malformed apical buds had a protuberance at the base of the buds which was absent in normal bud (Fig. 4.3a–c). Pandey et al. (2002) also confirmed that apical and auxillary buds with several protuberances at their base

Fig. 4.3 Initial stage of development of healthy and malformed buds: healthy vegetative buds (**a**), malformed vegetative (**b**), and floral buds (**c**) with protuberance at the base

gave higher proportion of malformation. The incidence of malformation was more in buds with increasing diameter. Length of buds did not show any correlation with incidence of floral malformation.

The fully developed inflorescences are with flowers (Fig. 4.4a) or with scale leaves or with both intermixed (Fig. 4.4b). The affected panicles continue to bear

Fig. 4.4 Inflorescence with flowers (**a**), and with flowers and scale leaves (**b**), healthy (*H*) and malformed (*M*) inflorescences from the same branch (**c**)

Fig. 4.5 Malformed panicle: transformation of flowers into leafy structures (**a**), fruit from malformed panicles (**b**)

flower buds even after fruit setting in the normal panicle is over. Malformed panicles hardly bear any fruit and, if at all, the fruits usually drop down before they attain pea stage. The peduncle becomes thick, green, fleshy and branches profusely. Repeated branching and continued formation of leafy growth makes the panicle formidable in size and weight that may be several times that of the normal one (Fig. 4.5a). Both healthy and malformed inflorescences often appear at a single growing point or on the same branch (Fig. 4.4c). At times parts of inflorescence become vegetative giving out small leafy structures (Fig. 4.5a). Each group of leafy structure represents a single malformed floret of the inflorescence. Some of the panicles that are initially malformed may recover as the inflorescences reach maturity and bear fruits (Fig. 4.5b). Many variations in the shape and form of malformed panicles have been observed on the degree of hypertrophy produced. These include:

- **Compact form—**In the compact form, the panicle is much stunted, the peduncle is thick and short with secondary branches crowded closely on it. In other cases, the peduncles attain normal size but secondary branches are arranged more

compactly so that instead of a single head there are a number of separate compact branches on same panicle (Fig. 4.6a).

- **Loose form—**In the loose form, the panicle is large in size and open in shape. The main and secondary branches put up further growth and produce scaly leaves. In such a panicle, the normal flowers are suppressed and replaced by tiny leafy structures or scaly leaves; the peduncle and its main and secondary branchlets are much thickened and the whole mass takes the shape of a 'witches broom' (Fig. 4.6b).

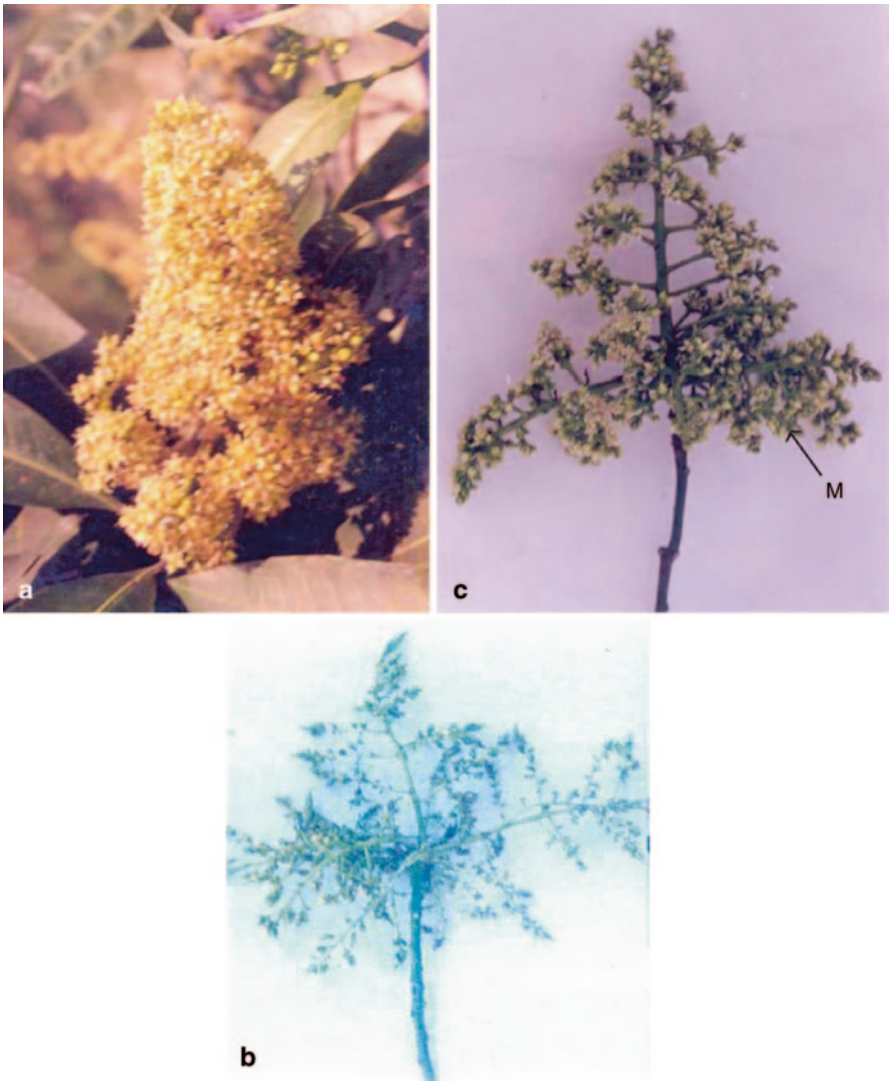

Fig. 4.6 Different forms of malformed panicles: compact (**a**), loose (**b**) and intermediate (**c**) (malformed = M)

Fig. 4.7 Dried and necrotic malformed panicle—site of multiplication of the pathogen

- **Intermediate grade—**In addition to the above mentioned forms, intermediate grades from a completely healthy to a totally malformed panicles are also found. In an otherwise healthy panicle, a few of its branchlets may be diseased (Fig. 4.6c).

During summer, most of the malformed heads dry up and undergo rotting during rainy season; thus, transformed into a black mass (Fig. 4.7) which persists on the trees for quite a long time. In addition, floral malformation having enlarged floral buds intermixed with leafy growth that can be seen occasionally even during winters.

The salient features of floral malformation are:
- Short internode, thickened peduncle
- Frequent transformation of flower buds into vegetative buds
- Less hermaphrodite flowers and viable pollens
- Prolonged longevity of the panicles

Flowers

The flowers are arranged more compactly along the axis. Individual flowers (Fig. 4.8a) in malformed panicles are hypertrophied, greatly enlarged, with a large disc, and the peduncle bearing them is also much thickened and look much greener (Fig. 4.5a). Hifny et al. (1978) observed that malformed panicles produce more flowers but most of the flowers of malformed panicles remain unopened (Fig. 4.5a).

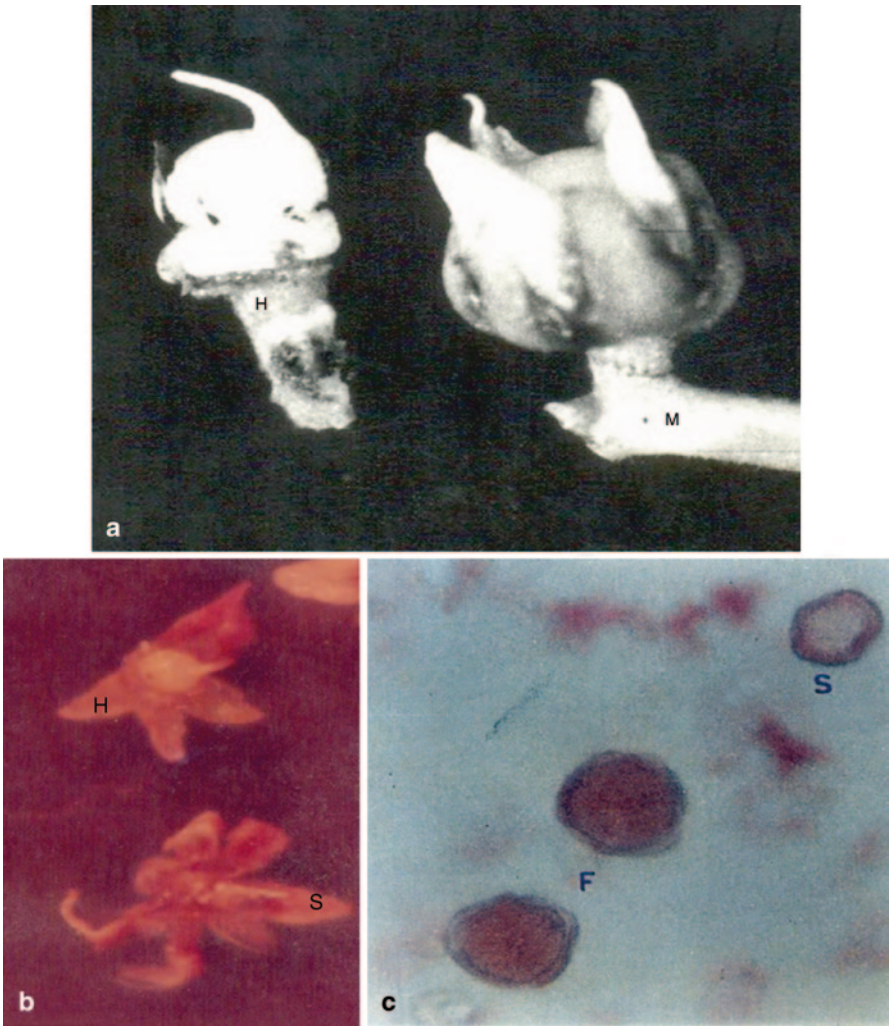

Fig. 4.8 Healthy (*H*) and hypertrophied malformed (*M*) flowers (**a**), hermaphrodite (*H*) and staminate (*S*) flowers (**b**), and fertile (*F*) and sterile (*S*) pollens

Mallik (1963) reported that the pistils in malformed hermaphrodite flowers are usually non-functional while Singh and Dhillon (1990) observed hypertrophied development of stigma, style and ovary of the affected flowers (Fig. 4.8a). Percentage of hermaphrodite flowers in malformed panicles is very low (Fig. 4.8b) and bears scanty pollen. Further, the pollens exhibit poor viability (Fig. 4.8c) (Mallik 1963). The hermaphrodite malformed flowers have one to four ovaries per flower as against one to two in normal ones (Singh and Dhillon 1990). These flowers show high degree of embryo degeneration. Singh and Dhillon (1990) noticed development of a thick and unorganized wall around the ovules that may deprive the ovule

of essential nutrients and metabolites in a developing fruit resulting into its degeneration.

Roots

Root system is also affected. Malformed seedlings usually have shallower root system with fewer tertiary roots and often tap roots are twisted and necrotic (Schlosser 1971).

Internal Symptoms

Several workers worked on the internal anatomical changes in relation to malformation through histopathological studies and steps in microsporogenesis.

Histopathology

Narasimhan (1954) observed that in malformed inflorescence, the anatomy of the cortex and stele is considerably transformed accompanied by the development of hyperplastic cells. The proliferation of host cells was also noted by Desai et al. (1962). Mallik (1963) reported that the pith cells are deformed and packed with starch.

However, elaborate information on histopathology of malformed affected plant parts was first published by Prasad et al. (1965). In malformation of vegetative types, both on seedlings as well as on young grown-up trees, there did not appear to be any difference in the internal structure of the branches bearing leaves with the normal ones. The deformation was confined to leaves only wherein the vascular tissue was very poorly developed and the palisade was conspicuous by its absence. The hypodermal and pith cells are filled with brownish fluid (Fig. 4.9). The brownish

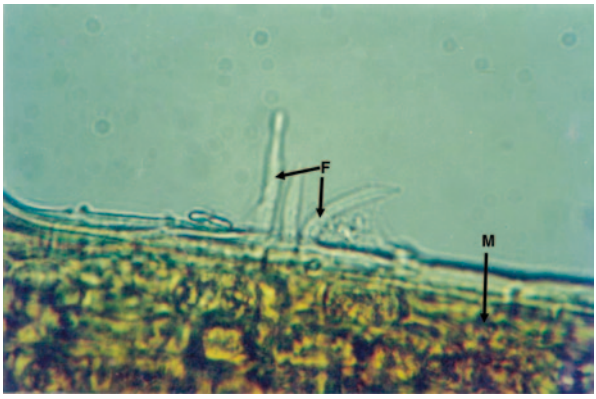

Fig. 4.9 Accumulation of brown fluid (mangiferin, *M*) at the site of infection by *F. moniliforme* var. *subglutinans* (*F*)

fluid present in diseased cells appeared to be very corrosive. In floral malformation the deformities were confined not only to the flower buds but extended to pedicels also. Their surface turned warty and puckered and a dense growth of epidermal hairs appeared on the surface. Other anatomical structures were almost same except that the affected ones contained much larger number of cells with brownish fluid.

Pandey et al. (1977) studied the anatomical changes in rachis of malformed panicles. The thickness of malformed rachis (6.3 mm) in cultivar Dashehari was approximately twice that of the healthy one (3.2 mm). Furthermore, the number of cells per unit area of cortex, xylem vessels and pith was 1½ times less in malformed panicles than in healthy ones. But the size of the cells of epidermis, cortex, xylem vessels and pith in case of malformed rachis was greater than those in healthy ones. The thickness of rachis and the smaller number of cells/field in the rachis of malformed panicles were a function of cell size.

Ibrahim and Foad (1981) investigated histology of malformed inflorescence and shoots. In the main axis of the malformed inflorescence, cortex, vascular tissues and pith cells were bigger in diameter. In the secondary branches of the malformed inflorescence, cortex is thicker and there is larger number of conducting elements; its peripheral zone is filled with tannins. In the normal branches, such zone is thinner and is restricted to a narrow strip.

The malformed shoot apex is distorted and lack the dome shaped appearance. The abnormal shoot apex produces a bigger number of conducting tissues. The component tissues of malformed blades are ill defined due to brown fluid, which is filling the ground tissues. Midribs of the malformed leaflets are poorly developed and difficult to delimit the outline of xylem vessels since they are partly blocked with a deeply stained substance. The outline of petiole is totally distorted; resin ducts accompanying the phloem tissues are of bigger size and number of vessels are less.

The salient internal features are:

- **In malformed inflorescence**
  - Bigger cell size
  - Cortical cells are filled with brown fluid
- **In malformed leaflets**
  - Xylem vessels poorly developed, lesser in number; blocked with brown coloured tannin

Anomalies in Microsporogenesis

During cytological studies (Kumar and Chakrabarti 2001), various types of meiotic anomalies were observed. The spindle fibers were lightly stained with aceto-carmin solution. In malformed buds, two to four chromosomes were found lying away from the metaphase plate instead of being attached with the spindle fibers by their centrosomes at the equator of the spindle (Fig. 4.10b). In case of malformation,

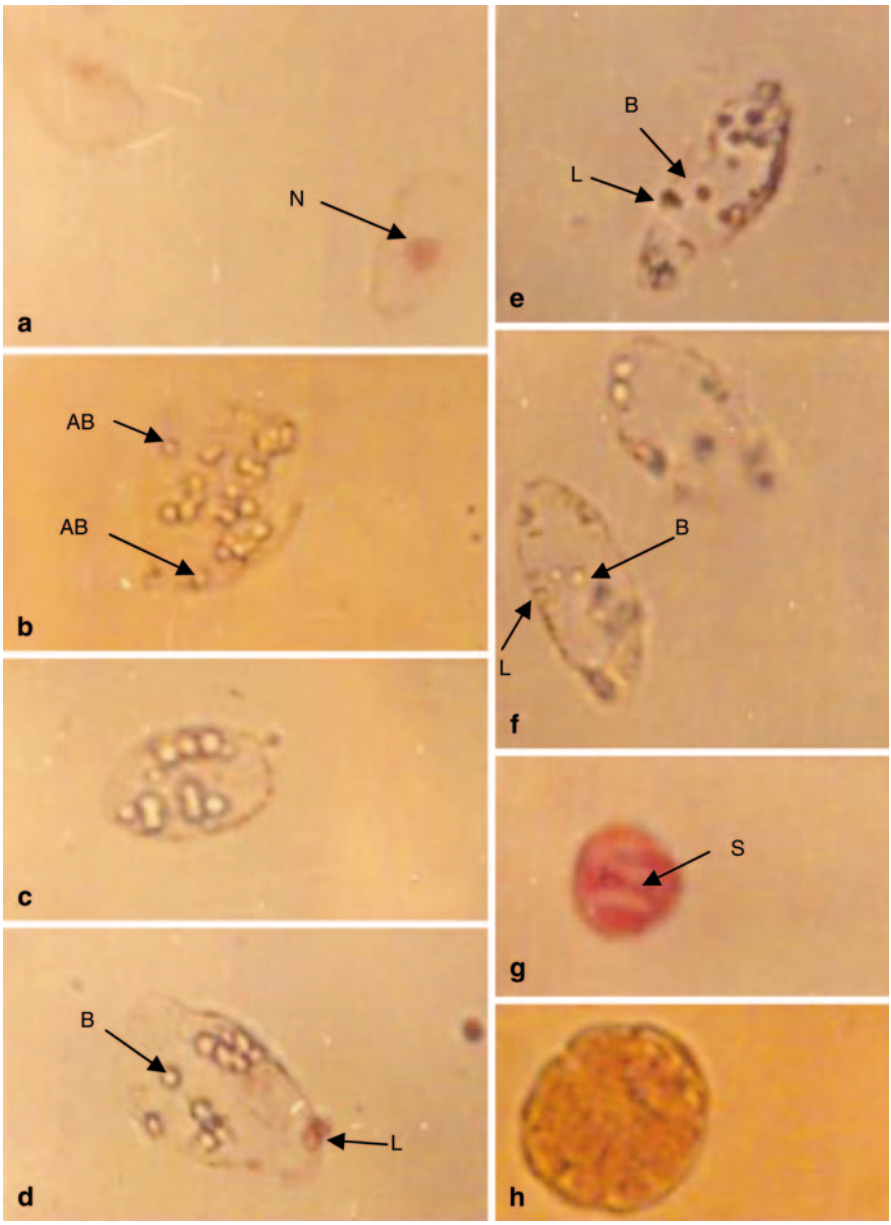

Fig. 4.10 Meiotic anomalies in *Mangifera indica.* **a** PMC with big nucleus (*N*), **b** Metaphase showing bivalents (*AB*) lying away from the plate, **c** Anaphase with reduced number of chromosomes, **d** Anaphase with 2 laggards (*L*) and one bridge (*B*); **e–f** Telophase showing bridge (*B*) and laggards (*L*), **g** Cytokinesis showing 3 separate and parallerl spindles (*S*), **h** Polyad

at anaphase, regular disjunction of homologous chromosomes did not take place. Instead, there were frequent occurrence of bridges and laggards both at anaphase (Fig. 4.10d) and telophase (Fig. 4.10e, f). In malformed buds chromosome number was reduced gradually from anaphase to telophase. During telophase further degeneration of chromosomes along with other organelles like cell wall was seen (Fig. 4.10f). Spindle anomalies were frequently noted at second meiotic division showing three separate parallel spindles (Fig. 4.10g). Subsequently, cytoplasm within the pollen mother cell (PMC) was divided by cell plate formation leading to the formation of polyads (Fig. 4.10h). It appears from the the circumstantial evidence that specific gibberellic acid, produced by *F. moniliforme* var. *subglutinans* (discussed in Chap. 5, p. 36), might be responsible for inducing chromosomal anomalies resulting in abortive pollens.

Chapter 5
Cause

Physiological Disorder

In analyzing causes, several physiological parameters were considered to be of importance at different times.

Carbohydrate and Nitrogen Ratio

Mallik (1959) estimated higher levels of carbohydrate, nitrogen and C/N ratio in malformed panicles. He (Mallik 1961) observed that in some trees setting of fruits in malformed panicles occurs during rains or later and the fruits ripened in November–December or even in January–February. He concluded that very high starch and nitrogen contents were mobilized into affected panicles during rains when new growth started. At the right stage of C/N ratio in those affected shoots, the pollen and stigma became viable, pollination occurred and fruits set. Not only the malformed panicles, even the shoots of mango trees destined to bear malformed inflorescence indicated a greater accumulation of starch than those which bear healthy inflorescence (Hamid 1960). Higher total sugar and starch content in malformed inflorescence as compared with the healthy ones was reported (Khan and Khan 1963). They also recorded significantly lower levels of nitrogen. The changes in carbohydrate reserves and in total nitrogen of healthy and malformed shoots of mango cvs. Dashehari and Chausa before and after fruit bud differentiation were recorded (Pandey et al. 1977). At both the growth phases (before and after fruit bud differentiation), levels of non-reducing, reducing and total sugars were higher in leaves of healthy shoots than the malformed ones. However, acid hydrolysable polysaccharides that favour induction of floral primordials remained at higher level in malformed shoots. In healthy stem and leaves, quantity of acid hydrolysable polysaccharides declined after fruit-bud differentiation. But in malformed leaves, stems and panicles its level remained higher even after fruit-bud differentiation. Two plausible explanations for the above phenomenon were suggested. First, presumably hydrolysable polysaccharides in malformed panicles

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were not hydrolysed into simple sugars in the same manner as they are hydrolysed in normal panicles for their development to meet the energy requirement. There may be certain pathogen involved in causing disturbance in metabolism. Secondly, it was observed that level of total carbohydrates decreased in the leaves with a corresponding increase in the stem. This fact indicates the mobilization of carbohydrate from the site synthesis to the site of utilization, perhaps for fruit-bud differentiation. But number of cells per unit area of cortex, xylem vessels and pith was ½ times less in malformed panicles than in healthy ones which might have impeded the transportation of photosynthates from leaves to the growing points. The nitrogen content in stems, leaves and panicles of healthy shoots, however, was higher. Thus, the carbohydrate and nitrogen ratios in malformed shoots were higher than in healthy ones in both the cultivars. In malformed leaves, higher amount of starch and reducing sugars and lower levels of total nitrogen were recorded (Chakrabarti et al. 1990). Higher levels of reducing sugars in malformed panicles and shoots were also noted by El-Ghandour et al. (1976). But Singh and Dhillon (1989b, 1993) reported higher nitrogen but lower carbohydrate contents in malformed tissues than the healthy ones. Babu-Koti and Rao (1998) observed that accumulation of starch remained confined to pith cells of shoots. The starch deposits started accumulating in September and attained maximum deposition in February. During summer and rest of monsoon months, there was no accumulation of these deposits.

However, Singh and Rathore (1983) recorded that the contents of reducing, non-reducing and total starch content at 30 and 50 days after anthesis in leaves and panicles of healthy and malformed shoots of Chausa were reduced in tissues of malformed trees compared with the healthy ones and the reduction increased with tree ages. Similarly, Singh (1986) also reported that in malformed panicles carbohydrate content as well as C/N ratios was also low throughout the developmental stages of panicles except at initial stage when buds were just swollen. But non-reducing and reducing sugars were higher. Contrary to malformed panicles, in malformed seedlings the levels of starch, total sugars and nitrogen content were higher. The reports by various investigators on levels of carbohydrates and nitrogen in healthy and malformed tissues although contradictory but they all point out towards a serious imbalance of C/N ratio in malformed cells.

Nutrient Levels

Chemical analysis of healthy and malformed leaves and stems showed that nitrogen percentage was marginally higher in malformed tissues over healthy. In the panicles, the same was more in healthy inflorescence. The picture was reverse with contents of potash and phosphorus (Tripathi 1955). Singh and Dhillon (1993) recorded low amount of phosphorus in malformed tissues than healthy ones. Leaf tissue analysis of mango revealed that the leaflets of malformed seedlings had the lowest levels of micro elements (Zn, Co, Mn, B) (El-Ghandour et al. 1979) as

compared with healthy leaves. Martin-Prevel et al. (1975) identified zinc deficiency to cause severe malformation in mature mango trees and the secondary symptoms to be associated with copper deficiency. In malformed shoots of cv. Amrapali both Zn^{++} and Cu^{++} were found at lower concentrations (Chakrabarti and Ghosal 1989). Abdel-Mottaleb et al. (1983) tested soil samples from root zones of malformed and normal trees. Recently Shah et al. (2009) estimated quantity of chromium, cobalt, cadmium, lead and magnesium in healthy and malformed mango buds and emerging panicles. They did not find any significant differences in their concentration.

The occurrence of panicle malformation was attributed to a decrease in the availability of copper and zinc contents in subsurface soil layers, especially on sandy soils, and or poor soil. Edward (2010) reported that manganese induced iron deficiency in mango plants which predisposes them to the infection.

During the investigation on trace element requirement of *F. moniliforme* var. *subglutinans,* the maximum growth was at 0.25 ppm of zinc. Iron, copper and manganese also promoted growth. Best sporulation was obtained in iron followed by zinc (Chattopadhyay and Nandi 1975). El-Ghandour et al. (1979) presumed that the trace element deficiency in malformed tissues might be the result of the *Fusarium* infection. Martin-Prevel et al. (1975) viewed the severe malformation in mature mango trees were due to deficiency of zinc while the secondary symptoms were associated with copper deficiency. Chattopadhyay and Nandi (1977a) observed accumulation of total phosphate content at the site of inoculation with *F. moniliforme* var. *subglutinans.* There was a gradual increase in phosphate content with advancement of the disease. But Tripathi (1955) did not observe any typical deficiency symptoms for nitrogen, phosphorus and potash on malformed plants. Chakrabarti et al. (2003) recorded the disease severity to increase proportionately with depletion of manganese content in the plants. Mn^{2+} -ions was demonstrated earlier to enhance formation of H_2O_2 which is anti-pathogenic and induces the host resistance (Mader et al. 1980). Kumar (2008) reported that *F. moniliforme* var. *subglutinans* produces copious amount of protease both *in vitro* and *in vivo*. When mango leaves were treated with crude enzyme preparation of the protease, it damaged the leaf membrane severely with concomitant loss of electrolytes. Similar electrolyte loss was recorded in malformed leaflets. Thus, it was proposed that the damaged cell wall permeability might be a contributing factor to the apparent micronutrient deficiencies in malformed plants.

Hormonal Imbalance

The disease symptoms and physiological changes inside infected tissues suggest that a hormonal imbalance occurs in the malformed shoots and panicles. The symptoms observed in malformed shoots and panicles showed similarities to the external manifestations of effects of different phytohormones recorded elsewhere by various scientists. The analogies lead the scientists to consider mango malformation as a physiological disorder more precisely a hormonal imbalance. Some of the

Tab. 5.1 Symptoms of mango malformation and their probable link with qualitative and quantitative changes with growth hormones

Symptoms of mango malformation	Associated hormonal changes as recorded in other plants	References
1. Stunting of shoots and panicles	Decreased level of gibberellins	Elstner (1983)
2. Loss of apical dominance	Higher level of cytokinin	Elstner (1983)
3. Prolonged longevity of malformed panicles	High NAA or GA or amino acid content; NAA and GA retard senescence	Penel et al. (1985)
4. Shifting of flowering buds towards vegetative growth	Either very high or very low amount of auxins at vegetative tips	Tanimote and Harada (1985)
5. More staminate flowers	High gibberellins or ethylene content	Lieberman (1979)
6. Pollen sterility	More gibberellins	Nelson and Rossman (1958)
7. Increased cell size	More gibberellins that generally lead to increase in plasticity and induce growth and wall synthesis	Nakumara et al. (1975)
8. Low transpiration vis-à-vis higher moisture content	Abscisic acid	Nickell (1982)
9. Disease resistance	Continuous presence of auxins at high level stimulates ethylene synthesis that in turn produces phytoalexins, the defense chemical of plants	Lieberman (1979)

important symptoms of mango malformation and effect(s) of phytohormones on different plants are listed in the following Tab. 5.1.

Several workers compared hormonal status of healthy and malformed panicles at different stages of their development both qualitatively and quantitatively. The imbalance between the growth promoters and inhibitors as recorded in the diseased shoots or panicles was attributed as the primary cause of the disease. Later experimental evidences suggested that the hormonal imbalance was the consequence of the host-parasite interactions.

Auxins

One group of scientists recorded lower amount of auxins but higher levels of inhibitors in healthy as compared with malformed panicles. Jagirdar and Jafri (1966) suggested that the disorder is related to the imbalance of auxins and anti-auxins in the plant at the time of floral differentiation and may be caused by factors such as pests, diseases and nutritional deficiencies. Majumder et al. (1970) also endorsed the above hypothesis and assumed that number of hermaphrodite flowers in malformed panicles might be due to depletion of auxin. They applied naphthyl acetic acid (NAA) (200 ppm) at the first week of October and got reduction in malformation. Subsequently the imbalance between growth promoters (auxins) and growth

inhibitors (not specified) which was in greater quantity was also confirmed by Pandey et al. (1974). They recorded free neutral, free acidic, bound neutral and bound acidic fractions of auxins of healthy and malformed buds (at bud burst stage, 2 cm long) of Dashehari. The levels of all fractions of auxins were higher in healthy buds as compared to malformed ones; the levels of inhibitors showed a reverse picture in two types of buds. Since there is an imbalance between growth promoters and growth inhibitors, it resulted in the suppression of apical dominance and made the developing buds malformed. Kumar et al. (1980) detected two growth promoting zones in healthy but only one in bunchy top (BT) affected seedlings. IAA and IAN (3-indoleacetonitrile) decreased by 98.4 and 92.6% respectively in BT affected tissues. Even the shoots carrying malformed panicles contained lower levels of endogenous auxins than those carrying normal panicles (Dahasan 1987).

On the contrary, higher levels of auxins have also been recorded in malformed shoots by several workers. Abou-Hussein et al. (1975) studied activity of auxins in the extract of axis of developed panicles of cv. Timour. The axis extract from normal panicles was found to contain three defined inhibitory zones. But axis extract of malformed panicles only two distinct promoting zones existed. The net units of promoting and/or inhibition revealed the shift from inhibition noted in axis extracts of normal panicles towards promotion in those of malformed ones. He argued that auxins at high level stimulate maleness. Rajan (1986) observed that in malformed panicles activity of acidic auxins was higher at balloon stage, but it remained lower than the normal one at rapid growth phase of the panicle and increased at later stage. Non-acidic auxins were 50 times more in malformed panicles at 48 days after bud burst. Chakrabarti et al. (1990) recorded higher amount of 3-indole-acetic acid in malformed shoot apex.

The depletion of auxins or increase in quantity of inhibitors was found to be linked with the *Fusarium* colonized in the host cells. Kumar et al. (1980) did not detect any growth promoting zone in the culture filtrate of *F. oxysporum;* on the contrary two very strong inhibitory zones were present. They suggested that the inhibitors detected in BT-affected tissues were of fungal origin. The activity of IAA oxidase in healthy and BT-affected seedlings was 2 and 9 units respectively. The enzyme activity in culture filtrate was 18.5 units. This was interpreted to show that the fungus could produce IAA oxidase both *in vitro* and *in vivo*. Loss of apical dominance and proliferation of buds with production of small leaves in affected seedlings could be attributed to (1) considerably decreased IAA as a result of oxidation of IAA oxidase which seems to be of the fungal origin and (2) accumulation of increased amount of inhibitors which also seem to be of fungal origin. Pal et al. (1983) also recorded greater IAA oxidase activity in malformed panicles than in healthy panicles. Contrarily, Chakrabarti et al. (1990) recorded lower activity of IAA oxidase in malformed shoots.

Gibberellins

Likewise, levels of gibberellins in malformed tissues have been reported both as high and low.

Abou-Hussein et al. (1975) estimated higher biological activity of gibberellins in the axis extracts of malformed panicles. The net units of promotion and/or inhibition for extract of normal panicles were changed from minor inhibition to considerable promotion of those of malformed ones. In malformed panicles, promotion of bracts, growth and high initiative towards branching may be attributed to the high levels of auxin and gibberellins activity. It is also well known that GA_3 induces maleness in large number of plants. Mishra and Dhillon (1980) extracted gibberellin-like compound from panicles of Dashehari. Healthy panicles showed 170.39 µg/g of GA_3 equivalents as compared to 254.94 µg/g in the malformed panicles. Increased levels of a GA_3 may account for the production of male flowers, continuous growth and persistence of malformed panicles on the tree. Ram and Bist (1986) estimated gibberellin(s) content in malformed panicles was generally at higher levels than in healthy ones. They recorded the pattern of changes in gibberellins content of developing malformed panicles was different from those in healthy panicles. They concluded that increased cell size, as was reported in malformed mango panicles, was caused due to the higher levels of gibberellins.

On the contrary, El-Ghandour et al. (1976) recorded low level of endogenous level of gibberellins in malformed tissues. In BT-affected tissues either none or only low level gibberellins-like substances were detected, whereas these were easily detected in healthy tissues (Kumar and Beniwal 1992). Rajan (1986) observed depletion of gibberellins in malformed panicles at rapid growth stage. Ram and Bist (1986) also recorded higher levels of gibberellin(s) content in malformed flower buds only at very early stage of its growth. Dahasan (1987) recorded lower levels of gibberellins in shoots carrying malformed panicles than in those carrying normal ones.

The qualitative and quantitative changes in gibberellins in malformed tissues have considered due to the extracellular gibberellins secreted by the *F. moniliforme* present in malformed panicles (Ram and Bist 1986).

Fluctuation of Auxins and Gibberellins in Malformed Cells The developmental stages of buds appear to affect nature and content of auxins and gibberellins in malformed panicles and shoots (Kumar and Beniwal 1992). Besides, Chakrabarti et al. (1997a) and Babu-Koti and Rao (1998) observed the population of *F. moniliforme* var. *subglutinans* in and outside of the host cells to vary in different season of the year as well as at different stages of growth of the malformed shoots and panicles. The fungus either produces itself or stimulates the host to produce the hormones or their inhibitors. Thus, the variation in the fungal population results into waxing or waning in quantity of auxins and gibberellins in malformed tissues and their concomitant effects.

Cytokinins

Ram and Bist (1986) reported that the malformed tissues not only contained higher amount of cytokinins but they differed qualitatively from that present in healthy materials. But the patterns of changes of cytokinin during growth stages of panicles

unlike that of gibberellins in healthy and malformed tissues followed a similar pattern. Rajan (1986) also observed higher amount of cytokinin in malformed panicles. A comparison of the cytokinin complement of healthy and malformed inflorescences indicates the absence of *trans*-zeatin (tZ), dihydrozeatin (DHZ) and ribosyldihydrozeatin (DHZR) in malformed flowers. Instead *Iso*-pentyladenine (2iP), not detected in healthy flowers, is produced abundantly in malformed flowers. Reduction in quantity of DHZ-like compounds which are required for normal flower development and fruit production seems to contribute to manifestation of malformation (Van Staden and Nicholson 1989). Similarly, Singh and Dhillon (1989a) recorded higher endogenous level of zeatin in malformed panicles and assumed this might be responsible for promoting cell division in malformed panicles resulting into its large size.

Ram and Bist (1986) assumed that the aberrant cytokinins that were detected in malformed tissues might be synthesized by the fungus, *F. monilforme* colonizing the malformed cells. Later Van Staden and Nicholson (1989) detected a number of cytokinin-like compounds mainly 2iP from the mycelia and culture media of *F. moniliforme* and also from the malformed flowers. In their opinion (1) production of 2iP by the fungus in host cells, (2) increased production of ribosyl zeatin (ZR) and glycosyl-O-zeatin (ZOG) and (3) blocking of synthesis of DHZ-like compounds in malformed flowers may be the reasons of malformation.

Ethylene

Singh and Dhillon (1990a) recorded higher amount of ethylene contents both in malformed panicles and shoots bearing malformed panicles and linked them to greater isodiametric growth of cells in the rachis. It was suggested that the higher level of ethylene in malformed panicles could be suppressing apical dominance of panicles, increasing isodiametric growth of rachides, and thickening of the secondary branches of malformed panicles producing overcrowded flowers. Endogenous ethylene is produced in the presence of either malformin or abscisic acid (ABA). Significantly higher levels of ethylene were detected in malformed panicles compared to healthy ones at the developmental stages. The increment of ethylene over the healthy panicles at different growth stages i.e. (1) fully swollen buds, (2) bud inception, (3) full grown panicle prior to full bloom and (4) full grown panicles and full bloom were estimated as 46, 145, 67 and 34% respectively (Pant 2000). Ethylene is a well known systemic signal molecule that activates defense genes in the host plants (Xu et al. 1994).

Abscisic Acid (Promoter/Inhibitor)

Mishra and Dhillon (1978) detected 58.32 μg/g of abscisic acid like substances in healthy panicles of Dashehari as against 31.58 recorded for malformed panicles. Malformed panicles showed lower inhibitors as compared to healthy panicles. In

healthy, only one against three promoting zones in malformed panicles were detected on chromatograms. The lowered inhibitor and increased promoter activity may possible account for continuous growth of the malformed panicles, their persistence on the tree and for production of mostly male flowers. High level of abscisic acid in BT-affected tissues as compared to corresponding healthy tissues has been observed (Kumar and Beniwal 1992). Ghosal et al. (1979) detected substantial amount of xanthophylls-like compounds viz. zeaxanthin and violaxanthin that are the precursors of abscisic acid in the culture filtrates of *F. moniliforme.*

Phenol

Site of Phenol Accumulation Initially Prasad et al. (1965) in a histopathological study, observed that the hypodermal cells of pedicels of malformed panicles contained a brownish fluid which was rarely present in healthy portions. Likewise, larger cells of the pith region and cortical cells in malformed shoots and panicles contained a brownish fluid more often than in the normal. Similarly, in malformed inflorescence of Taimour, the cortex of secondary branches had a peripheral zone of cells filled with tannins which constituted one half of the whole cortical thickness. In the normal branches, such zones were much thinner. In leaf, the component tissues of the malformed blades were deeply stained and ground tissues were filled with a brown fluid. In petiole, presence of the tannin-filled cells at the border or through vascular cylinder or pith were prevalent both in the normal and malformed petioles (Ibrahim and Foad 1981). The pith cells of floral meristem and mesophyll of leaf primordia, the site of infection, had the high phenolic accumulation (Babu-Koti and Rao 1998). The fact that phenolic accumulation was due to infection by the pathogen was substantiated when inoculation of shoots of susceptible Taimour variety with either *F. moniliforme* or *F. oxysporum* resulted in a significant increase in the phenolic content of juvenile shoots (El-Ghandour et al. 1979). In panicles artificially infected with *F. moniliforme* var. *subglutinans,* the phenolic accumulation was detected in areas surrounding the fungus invaded cells (Chakrabarti and Ghosal 1989).

Estimation of Phenol Higher levels of total phenols were recorded both in malformed panicles (Maheshwari and Mukherji 1975) and shoots (El-Ghandour et al. 1976). Drastic changes in nature of content of normal metabolites were observed in extractives of malformed flowers. Gallic acid, 4-methyl gallic acid and glucosamine were depleted in response to the infection; galactosamine remained unaffected, and mangiferin content was increased many folds (Ghosal and Chakrabarti 1984). In a further study (Ghosal et al. 1978b; Ghosal and Chakrabarti 1988; Chakrabati and Ghosal 1989) the qualitative and quantitative estimation of phenolic compounds in healthy and malformed panicles were reported in details. The malformed inflorescence at an early stage of infection afforded 4-O-methylgallic acid in very high yields. The compound was present only in traces in healthy inflorescence. In healthy

florets, mangiferin, the major phenolic constituent of young leaves and bark of *M. indica,* was either absent or present in only in trace quantities. Healthy florets, on the other hand, produced appreciable amounts of esters of glucose and gallic- and hexahydroxydiphenic acids (gallo- and ellagi-tannins). The amount of these compounds rapidly increased with the growth of the florets. Additionally, healthy florets were found to contain a new phenolic amide. Gallic and hexahydroxydiphenic acid conjugates were present only as minor entities in both malformed and artificially infected florets, at any stage. Appreciable amounts of brown polymeric quinine, 4-O-methylgallic acid, instead of the gallo-and ellagi-tannins, were isolated from the diseased florets. Whenever the species was afflicted with any form of stress, e.g. cut injury or infection by pathogen, magiferin and 1,3,6,7-tertrahydroxyxanthone were produced and accumulated in the injured organs. These entities and gallic acid conjugates, present in the infected florets as minor components, in turn, were oxidized into polymeric quinones. Presence of mangiferin, elagic acid, gallic acid and galloyl derivatives of glucose both in healthy and malformed shoots was confirmed by Rajan (1986). He isolated more phenols in flowers than rachis. The level of mangiferin was lower in panicles than in the vegetative shoots and leaves. Further two flavones, tetrahydroxy (Kaemferol) and pentahydroxy (quercetin) were detected in flowers of panicles. In malformed shoots, levels of total phenol and ortho-dihydroxyphenols were higher than the healthy ones (Singh 1986). Phytochemical studies revealed that compounds, such as, flavonoid, quercetin, mangiferin and phenolic acids like syringic, gallic and ferulic acids and proanthocyanidin and aponin were more in diseased panicles. P-hydroxybenzoic acid and phenol were absent in healthy panicles. This might be due to post-infectional effects which helped to increase these compounds in malformed panicles (Rao et al. 1996).

Role in the Disease It was observed that whenever the species was afflicted with any form of stress, e.g. cut, injury or infection by a pathogen, magiferin and 1,3,6,7-tertrahydroxyxanthone were produced and accumulated in the injured organs (Ghosal and Chakrabarti 1988). Both are potent fungistatic agents. Thus, further ingress of the the *Fusarium* into the host organelles was prevented. These entities and gallic acid conjugates, present in the infected florets as minor components, in turn, were oxidized into polymeric quinones. The polymeric quinones caused collapse of the adjoining cells thereby removing the source of nutrition and of multiplication of the fungus. The role of mangiferin has been discussed under the Chap. 5 ‘Mangiferin’. Mallik et al. (1986) reported that gallic acid inhibits the acivity of amylase which seems to be responsible for higher levels of starch content in malformed tissues. Phenolic substances have been demonstrated to act as growth regulators through IAA oxidase interaction and as gibberellin(s) antagonists. It is believed that these compounds play a major role in modifying the growth habit of healthy and malformed tissues (El-Ghandour et al. 1979). The synthesis and accumulation of phenolic compounds has been reported to impart resistance to diseases in plants (Wada et al. 2001). It was recorded that on inoculation of shoots of susceptible Taimour variety with either *F. moniliforme* or *F. oxysporum* resulted in a significant increase in the phenolic content of juvenile shoots (El-Ghandour

et al. 1979). This increase was not so clear in juvenile shoots of resistant variety Zebda. Similarly, maximum accumulation of mangiferin was recorded in resistant cv. Elaichi while minimum in susceptible cv. Beauty Mc-lin. Mangiferin promoted vegetative growth and exhibited inhibitory role on the occurrence of malformation. Mangiferin could be considered as potential biochemical indicator for screening mango genotypes to malformation (Singh 2006). Dhillon and Singh (1989) sprayed catechol (100 ppm) prior to flower bud differentiation (first week of October) which significantly reduced malformation and improved yield. They also recorded reduction in malformation by treating with gallic acid and salicyclic acid. Thus, it was suggested that exogenous application of polyphenols or O-dihydroxypolyphenol to minimize destruction of IAA and increase in IAA level prevents the manifestations of malformation symptoms (Singh and Dhillon 1993). Quercetin is known to act as fungitoxic and growth promoting flavonoid. Flavonoids and proanthocyanidin have both growth enhancing and deleterious effects. The elaboration and accumulation of the phenolic amide only in healthy flowers is also physiologically significant. Amides of aromatic hydroycinnamic acids have been frequently found to occur and accumulate in hermaphrodite flowers. Such amides were found to be completely absent in staminate flowers. Elaboration and accumulation of the amides of hydrocinnamic acids would seem to be linked with physiology of flowering. They also play important roles in normal growth and protection of flowers as anti-viral and antifungal agents as well as antifeedants. Ibrahim and Foad (1981) deduced that presence of tannins has no bearing on normal or abnormal growth of the inflorescence as the same occurs both in healthy and malformed tissues. Rajan (1986) did not find the level of mangiferin in malformed panicles supra-optimal enough to cause abnormalities.

Mangiferin Mangiferin (1,3,6,7-tetrahydroxyxanthone-C_2-β-D-gulcoside) is a natural metabolite of mango. It is synthesized in leaves and remains stored at bark. During flushing, mangiferin is mobilized into the growing region and is utilized in forming new shoots and leaves (Chakrabarti and Ghosal 1985). Mangiferin being phenolic in nature also acts as a defensive chemical compound of the plant (Ghosal et al. 1979; Sen 1981). Besides it serves as the carrier molecule of micronutrients (Chakrabarti and Ghosal 1989).

Mangiferin is a pale yellow needle-like compound. Molecular weight of mangiferin ($C_{19}H_{18}O_{11}$) is 422 and its melting point 271°C; with ferric chloride it gives a positive test. The structure of the compound (Chaudhuri and Ghosal 1971) has been presented in the Fig. 5.1.

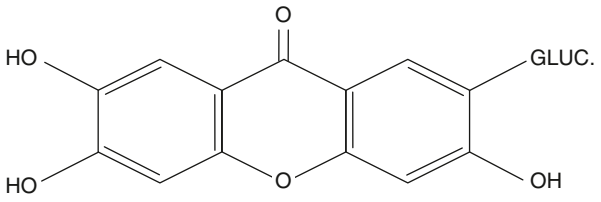

Fig. 5.1 Chemical structure of mangiferin

The quantity of mangiferin was estimated in different growth stages of healthy and malformed inflorescence of *Mangifera indica* L. var. Rajapuri (Chauhan and Rao 2000). More mangiferin was observed in malformed inflorescence at all stages of its growth i.e. bud stage, middle stage of bud growth and at full bloom stage. Similarly, higher mangiferin content was recorded in malformation resistant mango cultivars than the susceptible ones (Singh 2006).

The host plant produces mangiferin primarily to face challenges from abiotic and biotic stress like injury or invasion by parasitic microorganisms (Kishore and Syamal 2006). But excessive accumulation of mangiferin at the site of infection brings about a wide range of biochemical and physiological aberrations in the host cells which ultimately manifested the disease symptoms (Chakrabarti et al. 1990). Additionally it induces significant morphological and physiological changes in the pathogen, also gives rise to new physiological race (Kumar and Chakrabarti 1992, 1995). Thus, the biochemical weapon of the host seems to backfire. Knowledge of intrinsic role of mangiferin in the *F. moniliforme* var. *subglutinans-M. indica* pathosystem helps to unfold the enigma of mango malformation (Chakrabarti 1996; Chakrabarti and Kumar 2002).

Role of Mangiferin in Symptom Production Mangiferin has been shown to be involved in symptom production in various ways. Some major issues are discussed here under.

- **Mangiferin as suppressor of usual symptoms of *Fusarium*—**As soon as the pathogen invades the host, a large amount of mangiferin is accumulated in the cortical cells surrounding the invaded zone (Fig. 4.9) (Ghosal et al. 1979; Chakrabarti and Ghosal 1989; Rao et al. 1996; Chauhan and Rao 2000; Kishore and Syamal 2006; Singh 2006; Singh et al. 2007). Earlier workers (Ibrahim and Foad 1981; Prasad et al. 1965) also noticed the accumulation of brown fluid in the hypodermal cells of malformed leaflets and panicles but its chemical nature was not identified. Mangiferin prevents the pathogen to go deep into the host cells. Thus, *Fusarium* pathogen cannot reach the xylem vessels and spread systemically (Babu-Koti and Rao 1998). Occurrence of both healthy and malformed panicles on the same branch (Fig. 4.4c) corroborates the above contention. Mangiferin is antifungal in nature (Ghosal et al. 1977) and kills the pathogen in the invaded cells. This might be the reason of occasional failure to isolate the fungus from actively growing malformed shoots and panicles (Prasad et al. 1972). On living host cells *F. moniliforme* var. *subglutinans* could not sporulate. This is because mangiferin stops the breakdown of host starch (Chakrabarti et al. 1990). Starch is a compound required for growth and sporulation of the fungus (Mitra and Lele 1981). Mangiferin also arrests the production of fusaric acid by the pathogen (Ghosal et al. 1977, 1979). As a result, fusaric acid-induced symptoms like chlorosis, epinasty, vascular browning and wilting are not produced in malformed plants.
- **Mangiferin as inducer of unusual disease symptoms—**Leaves from the axils of which malformed panicles develop contain high amount of mangiferin

Tab. 5.2 Symptoms of mango malformation induced by accumulate mangiferin

Effects of mangiferin	Symptoms
1. Increased IAA content	More vegetative growth
2. Increased chlorophyll content	Malformed shoots/panicles look greener
3. Increased photosynthesis	More carbohydrate synthesis
4. Reduced respiration and amylase activity	Carbohydrate accumulation, disturbed C/N ratio
5. Reduced catabolism	More longevity
6. Reduced transpiration	High moisture content

(Chakrabarti and Sharma 1993). During active growth stage of the panicle, mangiferin from these leaves is translocated to the adjoining panicles tilting the hormonal balance. Mangiferin not only serves as a defense substance but like glyceollin and several other phytoalexins, acts as normal plant growth metabolite. Mangiferin increases chlorophyll content (Chakrabarti et al. 1990). Thus, malformed shoots and even the panicles look greener. Subsequently mangiferin increases the photosynthesis. On the other hand, it reduces the rate of respiration and amylase activity. Thus, the carbohydrate content of the malformed shoots and panicles becomes high. But as the transport system of malformed plants is disrupted due to immobilization of mangiferin at the site of infection, carbohydrate accumulates therein. Babu-Koti and Rao (1998) recorded gradual increase of starch accumulation parallel to the disease severity in the shoots of mango from September to February. The accumulation of carbohydrate disturbs the C/N ratio and induces large number of floral primodia. Mangiferin also increases auxin content. Thus, the high amount of mangiferin around the invaded cells stimulates the production of leaflets and shootlets and promotes the transformation of florets into leafy structures. Mangiferin affects the activity of the host enzymes viz., invertase, amylase, catalase and slows down the rate of respiration i.e. catabolic activity (Tab. 5.2). Besides, mangiferin is a cytokinin augmentation factor and promotes vegetative growth significantly (Ghosal and Chakrabarti 1988; Chakrabarti et al. 1994). Thus, it is responsible at least partially for the increased longevity of the malformed shoots and panicles and excessive vegetative growth. Mangiferin reduces rate of transpiration and consequently, moisture content of malformed panicles becomes high. Increased water content of malformed cells presumably contributes to defense against the colonization of the pathogen in the host cells (Bollard and Matthews 1966). But due to the presence of large quantity of inhibitors and the fungal toxins in the malformed shoots and panicles as mentioned in the earlier chapter, growth promoting activity of mangiferin could not be expressed properly resulting in development of large number of poorly developed shootlets and inflorescence (Fig. 5.2). Besides in mango, mangiferin acts as the carrier ligand for micronutrients. As mangiferin remains immobilized at the infection site, the transportation becomes disrupted causing deficiency of micronutrients at the malformed buds and affects its normal growth. Venkateshwarlu et al. (2001) reported mangiferin as an allelopathin and observed that mangiferin at

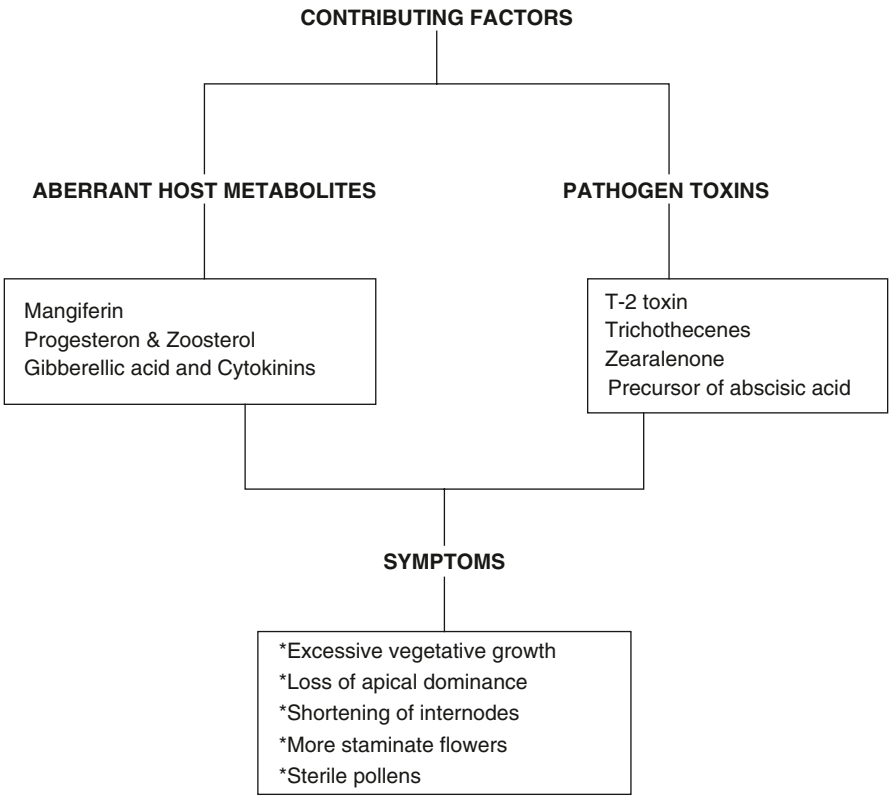

Fig. 5.2 Physiology of development of malformation symptoms

higher concentration (200 ppm) inhibited shoot and root growth of wheat and okra.

- **Interaction of mangiferin with the pathogen and its mite vector—**Population of *F. moniliforme* and *Tyrolichus. casei* are negatively correlated with mangiferin content (Chakrabarti et al. 1997). At the initial stage of disease development, a positive correlation is noticed. But with further increase in mangiferin content, the population of the pathogen declines. Mangiferin stimulates fungal growth at low concentration but becomes inhibitory at higher doses (Ghosal et al. 1977). The increase in the rate of symptom production is not affected till the *Fusarium* population drops down below the threshold level. The expression of the symptoms is affected due to lack of fusarial toxins. Thus, a proper balance of mangiferin, the fungal pathogen and mites is essential for development of the disease. In an experiment with cv. Banarasi Langra the disease was found to attain logarithmic growth phase and reached a peak (ca. 15% malformed panicles) when a balance was established among mangiferin content (35 mg/g), population of both *F. moniliforme* var. *subglutinans* (38 g^{-1}) and *T. casei* (1.2 g^{-1}) (Chakrabarti et al. 1997; Chakrabarti and Kumar 1998) (Fig. 5.3).

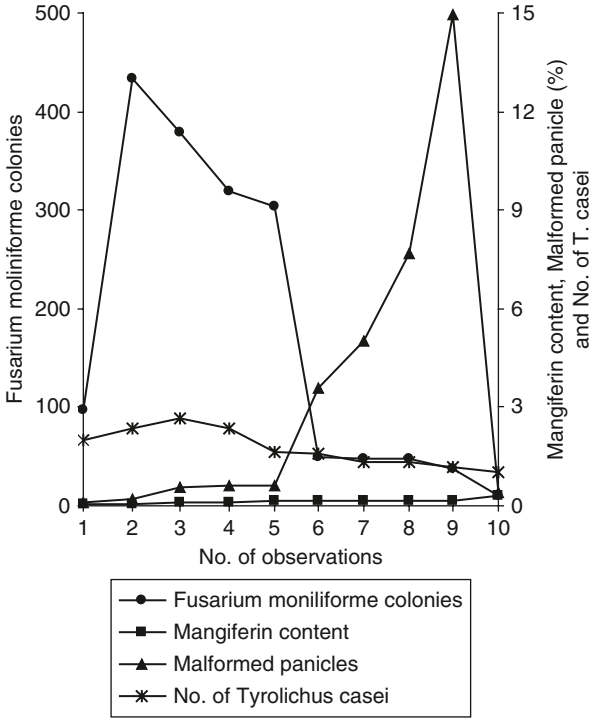

Fig. 5.3 Incidence of floral malformation in mango as related with population dynamics of *F. moniliforme, T. casei* and mangiferin content

Phytosterols

Ghosal and Chakrabarti (1988) recorded production and accumulation zoosteroids, pregnenolone and progesterone, instead of the normal phytosterols viz. acyl steryl glycosides, free sterols, steryl esters and steryl glycosides in the diseased flowers of mango. The qualitative shift in sterol contents seem to be associated with the impairment of the floral sex expression resulting in staminate, defective flowers with an emaciated ovary. The elaboration and accumulation of the phenolic amides only in healthy flowers are also physiogically significant. Amides of aromatic hydroycinnamic acids have been frequently found to occur and accumulate in hermaphrodite flowers. Such amides were found to be completely absent in staminate flowers. Elaboration and accumulation of the amides of hydrocinnamic acids would seem to be linked with physiology of flowering. They may also play an important roles in normal growth and protection of flowers as antiviral and antifungal agents as well as antifeedants. Phytochemical studies of Rao et al. (1996) revealed that compounds, such as, steroids were more in diseased panicles. This might be due to post-inflectional effects which helped to increase these compounds in malformed panicles. These compounds may play a key role in disease resistance of the host. Steroids and saponins are also known to have similar effects to that of sterols that are known to cause impaired floral sex expression.

Nucleic Acids, Amino Acids and Proteins

Sandhu (1975) reported that leaves from malformed shoots of cv. Langra contained greater number of and amount of free amino acids than the healthy shoots. He assumed that accumulation of free amino acids in the malformed leaves may be due to inhibition of protein synthesis resulting in reduction of leaf size. Similarly Chattopadhyay and Nandi (1977b, 1978a) observed that after inoculation of healthy shoots of Himsagar and Bombai, the protein, DNA and RNA contents increased in the course of infection. The increase in DNA content could possibly be attributed to the endomitotic duplication of chromosome threads leading to polytene condition and increased ploidy levels of the tissues. As a consequence of host-pathogen interaction, the increased DNA activity might be responsible for an increase in RNA content through rapid synthesis of certain RNA molecules or new RNA species. Singh (1986) estimated protein, total free amino acids, DNA and RNA in malformed seedlings as compared with the healthy ones as very high. But in malformed panicles the picture was just reverse. In malformed panicles the amount of protein and amino acids change with the stages of panicle development (Singh and Dhillon 1989b).

The malformed panicles had more proteins and total amino acids at bud inception and fully swollen stages whereas this trend was reversed in later stages. Majumder and Majumdar (1994) also observed that malformed shoots had higher protein, DNA and RNA content. Most of these constituents tended to decrease at the end of summer or during rainy season (Pandey and Narwadkar 1983).

On the contrary, Pandey et al. (1975) observed that RNA and DNA were higher in healthy panicles than the malformed ones. Soluble protein and amino acids were also higher in healthy panicles. Reduced levels of RNA, DNA and protein reflect their utilization in malformation of the large number of axillary buds to the detriment of apical buds. Mishra (1976) reported lower levels of free amino acids in leaves of malformed shoots of Dashehari. The healthy panicles contained higher levels of amino acids, RNA, DNA, soluble proteins and amides as compared to the malformed panicles. Chakrabarti et al. (1990) observed that contents of protein, DNA and RNA were more in growing tips of healthy mango shoots of Amrapali.

Chlorophyll

The concentrations of chlorophyll a and b as well as that of total chlorophyll contents were found to be significantly higher in leaflets of malformed shoots (Mishra 1976; Chakrabarti et al. 1990) in the cv. Amrapali. Contrarily (Pandey et al. 1973) it was reported that the amount of total chlorophyll (a+b) was about four times more in the leaves of healthy shoots as compared to the leaves of malformed shoots of the same tree. Subsequently, Pandey et al. (1977) did not observe any difference in the contents of chlorophyll in the leaves of the shoots carrying healthy and malformed panicles. Chattopadhyay and Nandi (1978b) recorded that the total

chlorophyll contents in the leaves and shoots bearing malformed panicles was lower in comparison to shoots of healthy panicles. Pandey et al. (1973) assumed the depletion of chlorophyll content in the leaves of malformed shoots is associated with preferential movement of assimilates namely carbohydrates, and nitrogen fractions in the stem where they accumulate without being further utilized in the development process like flowering. Thus, the leaves of healthy shoots continue to act as a stronger energy trapping system while those of malformed shoots failed to do so. Hence they look pale yellow and sickly. On the other hand, the increase in chlorophyll content in leaves of malformed shoots was substantiated by the concomitant increase in rate of photosynthesis and higher levels of carbohydrate accumulation (Chakrabarti et al. 1990).

Enzymes

Chattopadhyay and Nandi (1976) found the activity of both peroxidase and polyphenoloxidase increased considerably when the shoots of mango cultivars Himsagar and Bombay Green were inoculated with *F. moniliforme* isolate from mango. Greater enzyme activity was recorded in the cultivar Himsagar which showed better field resistance against the disease. However, the enzymes seemed to be produced by the host as *in vitro* no such enzymes were detected. Kumar et al. (1980) estimated the activity of polyphenol oxidase (PPO) and peroxidase (PO) both in healthy and bunchy top (BT) affected seedlings. No PPO and PO activity was recorded in healthy seedlings. But in BT-affected seedlings considerable activities of both PPO (32.2 unit) and PO (117.6 unit) were recorded. The IAA oxidase activity was also high. The IAA oxidase seemed to be of the fungal origin. The activity of PPO and IAA oxidase were attributed to depletion of auxin in BT- affected tissues. Pal et al. (1983) estimated the activity of oxidative enzymes in four cultivars viz. Dashehari, Mallika, Taimuria and Benazir. The amylase and catalase activity in malformed panicles was lower as compared to the healthy ones. But its IAA oxidase activity was higher. Rajan (1986) recorded higher levels of IAA oxidase and PPO but lower levels of PO in malformed tissues than in healthy ones. He suggested that high level of phenolic accumulation in malformed tissues might have accelerated the activity of the oxidative enzymes. Singh (2006) noticed activity of the enzymes like PPO, PO and catalase was the highest in resistant cultivar like Elaichi as compared to malformation susceptible cvs. like Amrapli and Dashehari. Chakrabarti et al. (1990) estimated low level of catalase and amylase activity in malformed panicles. Lowering of amylase activity, increased level of carbohydrates and that of catabolic activity prolongs longevity of the malformed panicles. Invertase activity was increased resulting in depletion of sucrose content. PPO activity was enhanced; but PO activity was lowered. More significant was lowering of IAA oxidase activity. Presumably accumulation of the phenolic compound (mangiferin) lowered the IAA oxidase activity. This is substantiated by the presence of high level of IAA in malformed panicles. Wada et al. (2001) estimated the activity of polyphenol oxidase

viz. catecholase and cresolase in malformed tissues. The catecholase and cresolase have been reported to be responsible for *in vivo* synthesis and accumulation of phenolic compounds that impart resistance to diseases in plants. They further observed a strong inverse relationship between activity of catecholase and cresolase and malformation incidence and proposed to use PPO activity to screen mango varieties for resistance to panicle malformation. Sharma et al. (2001) recorded strong negative correlation between PPO activity and malformation incidence. Incidentally regular bearing cultivars that have higher incidence of malformation as compared to the biennial bearers show lower polyphenol oxidase activity.

Toxins

Earlier several investigators through chromatographic studies and bioassays recorded the presence of growth inhibiting factors in malformed tissues. The exact chemical nature of these compounds and their source of origin were reported by Ghosal et al. (1979). They isolated several fusarial metabolites e.g. 12, 13-epoxy-trichothecenes from the *F. moniliforme* var. *subglutinans*-infected mango tissues as well as in the culture filtrates of the fungus. But fusaric acid (FA), a normal metabolite of fusaria, was absent in the malformed shoots and panicles. The fungus, however, regained its ability to produce FA *in vitro* at the 4th successive sub-culture stage. Addition of mangiferin (1×10^{-5} M), just prior to the 4th sub-culture stage, again arrested formation of fusaric acid. However, in all the infected tissues of *M. indica,* the fusarial borne sesquiterpene and resorcyclic acid, macrolactone toxins, which are known to cause phytotoxicity, were present (Chakrabarti and Ghosal 1989). The culture fluid of the fungus caused complete abscission of tender mango leaves. The ability to cause abscission was not observed in the culture fluid treated with tetracyclic interpene. Xanthophylls e.g. zeaxantnin and violaxanthin, which are liberally produced by the strain of the fungus were practically absent in the interpene treated culture fluid. In view of the fact, that abscisic acid is derived *in vivo* from carotenoids e.g. zeaxanthin and violaxanthin, this observation would seem to indicate the role, at least in part, of abscisic acid in malformation.

Kumar (1983) also observed that the disease involves a malformation-inducing principle (MIP). The MIP could either be some infectious (pathogenic) agent or its metabolic product. It does not get transferred into scions from root stocks or vice versa through vascular union. It acts at the time of vegetative or floral bud induction and conditions the cells to produce malformed growth. There is another principle i.e. toxic principle (TP) produced by the malformed tissues. The TP is produced inside the infected tissues as a result of infection and is present only in malformed tissues. The amount of TP is higher in vegetative malformed as compared to floral malformed tissues. It exhibits toxicity symptoms as retardation of growth of the seedlings or inflorescence and in severe cases, drying of the seedlings or destroying the flowering ability of the trees; but cannot produce typical symptoms by itself. It is able to translocate downward to cause toxicity in leaves below the inoculation

point. When malformed tissue extracts are injected into healthy floral buds, three to four leaves down became shriveled. Where the injected buds produced panicles, these initially remained stunted but recovered later. Thus the fungus colonized while multiplying at the growing point gradual release MIP for an extended period of time. MIP may condition the cells to produce malformed growth. Once the exponential growth phase of the fungus is over due to depletion of nutrients, it produces toxic secondary metabolites (TP) that are translocated in plant and results in toxicity symptoms such as reduced growth and necrosis of malformed tissues, seedling necrosis and loss of flowering.

The role of malformin-like substances has been proposed (Ram and Bist 1984). Malformin, a cyclic pentapeptide (cyclo-D-cysteinyl-D-cysteinyl-L-valyl-D-leucyl-L-isoleucyl) has been reported to be produced by *Aspergillus niger,* which causes malformation of maize roots and bean plants (Curtis 1958). Through chromatographic studies they detected presence of malformin-like substances in ether extract of malformed panicles, whereas it was absent in healthy panicles. The extracts also tested for malformin-like activity by two bioassays, the corn root curvature test and the mung bean (*Phaseolus vulgaris*) growth test. The activity was detected only with ether extract of malformed panicles. Singh and Dhillon (1987) reported occurrence of malformin-like substances in malformed seedlings. The stem and root of malformed vegetative seedlings contain high level of malformin-like substances. Kumar and Ram (1999) reported that *F. moniliforme* produces malformin-like substances *in vivo* also. Kumar and Beniwal (1992) observed that the culture extracts of the fungus caused typical curling a stunting of maize and pea roots and formation of flap-like growth of at tip of wheat roots, but failed to induce malformation in bean plants, as observed by Curtis (1958). Hence, it is yet to be confirmed that the compound from *F. moniliforme* var. *subglutinans* that caused root malformation in maize and pea was the same as the one isolated from *A. niger*. Singh and Dhillon (1987) presumed that malformin-like substances in mango might induce the malformation through an ethylene mediated system i.e. it might have stimulated ethylene production that in turn inhibited auxin transport through affected plant tissues resulting into hormonal imbalance and ultimately leading to manifestation of the disease symptoms.

Virus and Mycoplasma-Like Organism

Sattar (1946) could not identify any insect pest that causes the disease or isolate any pathogenic organism from the malformed tissues. However, it appeared to him that nature of attack, spread of infection and rise in the incidence of the disease and its presence on both the seedlings and grafted varieties are like those caused by virual pathogen. Thus, he presumed that the disease might be of either viral origin or is a physiological disorder. Singh and Jawanda (1961) found close resemblance of the malformation symptoms with those of leaf hopper-transmitted yellows type of diseases, considered viral at that time and hence, he inferred that a virus is the

causal organism of mango malformation disease. He referred greater incidence of malformation in areas heavily infested with mango hopper to substantiate his hypothesis.

To confirm the virual nature of the disease, tests like sap inoculation and graft transmission were tried by several workers. Transmission of the disease by grafting of the healthy stock with diseased scion was reported by Ahmad and Sattar (1950). Kausar (1959) reported transmission of the disease through buds taken from the branches of a tree bearing malformed inflorescence and budded on the branches of a healthy mango, thus showing the virual nature of the disease. Mallik (1963) also transmitted the disease successfully by grafting or budding. He also suggested that transmission of vegetative malformation by dodder was possible. Nariani and Seth (1962) successfully transmitted the disease to healthy mango stocks in about 50% of the wedge grafts; thus, they presumed the involvement of a virus with the causation of the disease. Bindra and Bakhetia (1971) used scion shoots from malformed plants after eradication of its bud mites by spraying acaricides and also maintained the grafted tests plants in mite free conditions. Nevertheless, the disease developed on 56–67% of the grafted test plants. They speculated that the disease might be due to 'a graft transmissible pathogen (virus, fungus or mycoplasma) alone or a complex formed with other factors like mite/fungus toxins, micronutrient deficiencies etc.'

Contrarily, Sharma (1953) observed that the disease could not be transmitted by sap inoculation. Singh et al. (1961) tried the sap inoculation test and graft transmission (inarching, cleft grafting and bark patch budding) and observed that out of 336 inoculated plants, only three plants showed initial symptoms of vegetative malformation in early stages. Later on, these, however, developed into normal leaf producing shoots. Thus, the disease was not produced on any of the sap inoculated plants. The results of graft transmission tests from diseased seedlings to healthy seedlings also showed that the disease was not graft transmissible. Prasad et al. (1965) also attempted to transmit the disease by different propagation methods (inarching and budding) in different seasons but without any success indicating that the disease may not be due to any virus. Salama et al. (1979) mechanically inoculated fourteen species of plants, belonging to five families with crude sap extracted from malformed inflorescence and vegetative parts of mango plants but no symptoms were observed. Further, the results of the graft inoculation test indicated that disease symptoms were not transmitted from malformed shoots to healthy seedlings by stem grafting. Kumar and Beniwal (1992) grafted (veneer grafting) diseased scion on healthy rootstocks and vice versa but there was no symptom production on healthy part of either combination (Fig. 5.4) which suggests non-graft-transmissible nature of the causal agent. Singh et al. (1961) verified the presumption of Singh and Jawanda (1961) that the malady may be of virus nature, and that the insects, particularly those having sucking type of mouthparts act as the vectors for the virus. They collected the sucking insects viz. mango hoppers, mealy bugs, thrips and aphis from the malformed shoots and liberated 50 each of the arthropods on ten mango seedlings. The inoculations with mango hoppers, mealy bugs, thrips and aphis failed to produce the symptoms. The speculation of

Fig. 5.4 Development of healthy scion shoot on malformed root stock (*M*)

virus origin of the malformation was put to end when electron microscopy of thin sections of petals, leaf midribs and fine roots from malformed mango trees failed to reveal signs of any virual pathogen in phloem or xylem tissues (Kishtah et al. 1985b). Similarly, no virus particle could be detected in sap or thin sections of seedlings or trees with this disease. Treatment of malformed trees and seedlings with oxytetracycline HCL did not prevent or inhibit malformation. Culturing an extract of malformed tissues did not change the red colour of PPLO medium. Results were negative when such extracts were tested by ELISA with conjugated antisera of *Spiroplasma citri,* corn stunt spiroplasma and the causal agent of peach leaf roll and aster yellows. In a separate experiment, Kishtah et al. (1985a, b) also recorded that the disease was not transmitted by grafting from scion to rootstock or from rootstock to scion. Heat treatment at 50 and 60°C for varying periods did not free the bud woods from the malformation agent indicating that neither a virus nor a mycoplasma causes this disease. Thus, none of the published works indicates the possibility of any virus or mycoplasma-like organism to be involved in the causation of disease.

Mites

Mites Associated with Malformed Panicles/Shoots The association of mites (*Aceria mangifera* syn. *Eriophyes mangiferae* Sayed) (Fig. 5.7d) with malformation of mango was first reported by Hassan (1944) from Egypt. Later Tahar (1946) observed mites to cause both vegetative and floral malformation. In India, Narasimhan (1954) from Poona recorded for the first time the occurrence of a species of eriophyid mite in malformed shoots. The mites are present in great number in diseased mango inflorescence in the meristematic regions and tender portion of the peduncle. The anatomy of the cortex and stele of inflorescence is considerably transformed and accompanied by the development of hyperplastic cells. Further, he added that eriophyses mites are both intra- and intercellular and incite cell enlargement and rapid multiplication of undifferentiated types of tissues (Narasimhan 1959). Besides *A. mangiferae,* Singh (1955) observed two more species of mites *Typhlodromus* sp. (which he tentatively identified as *asiaticus*) and *Tyrophagus castelanii* Hirst causing malformation of vegetative and floral.

Inoculation Tests The inoculation test with the eriophyid mites to produce malformation symptoms was conducted. Puttarudriah and Channa-Basavanna (1961) introduced the eriophyid mites taken from malformed twigs to just sprouting leaf buds of healthy plants. The inoculated buds produced malformed symptoms. Singh et al. (1961) conducted inoculation tests with *A. mangiferae, Typhlodromous asiaticus* and *Tyrophagus castellenii* and reproduced malformation. However, according to Narayanan and Ghai (1961) both these species are predators on phytophagous mites; hence, these two predatory species could in no way be associated with the disease. Singh et al. (1961) were also not sure whether mites were solely responsible for the disease or they act as vectors. Narayanan and Ghai (1961) identified three species of predatory mites, *Typhlodromus rhenanus, T. nesbitti, T. rosanlali* belonging to the family *Phytoseiidae* and *Chaloetogenes ornatus* belonging to the family *Cheyletidae* in addition to *A. mangiferae* from the malformed plants. According to these authors, it was because of the predaceous mites that the population of the phytophagous mite, *A. mangiferae* did not increase higher. Nariani and Seth (1962), unlike Singh et al. (1961), considered that the mites play a direct role in causation of the malformation symptoms. They inoculated one-year-old seedlings of mango cv. Langra with mites collected from diseased or apparently healthy plants. The disease symptoms were produced in both the cases. Thus, they concluded that the eriophyid mites are capable of causing disease symptoms in mango seedlings irrespective of whether they are picked up from diseased or apparently healthy plants. The authors opined that the malformation might be due to the toxins secreted by the mites.

Mite Population and the Disease Incidence Desai et al. (1962) reported that by decreasing population of bud mites after spraying the plants with Folidol or Ekatin, the disease incidence was simultaneously reduced by 99% indicating role of the mites in disease incidence. Similarly, others observed that killing of the mite (*A. mangifera*) over the heavily infested terminal buds of the bunchy top shoots with

diazinon (0.1%) emulsion, the malformed saplings recovered and showed normal growth (Rai and Singh 1967; Yadav 1972). Reduction in malformation with the spray of diazinon was also reported (Singh 1956). The reduction in the disease incidence by using acaricides was considered an indirect proof of the involvement of mites with the disease.

Disprove of Role of Mites On the contrary, several workers ruled out the possibility of mite as a cause of malformation. Thus, Prasad et al. (1965), Prasad (1972) concluded that the disease is not the result of a direct injury by the eriophyid mite, *A. mangiferae*. In support of the contention, they put forward the following evidence: (1) The population of mites between the scales of terminal buds of healthy plants and malformed buds was compared and it was observed that there was not much difference in the population; rather the number of mites being slightly on a higher side in the terminal buds of healthy plants. It means that there is no correlation between the population of eriophyid mites and bud malformation. (2) No stage of eriophyid mites was observed between the scales of the swollen buds that are still compact. However, these swollen buds invariably turn out to be malformed. Thus, there seems to be no correlation between the eriophyid mites and bud swelling. This again shows that eriophyid mites enter the malformed buds only after the scales become loose. (3) The disease could not be controlled by use of any acaricidal spray even started before the bud burst stage. Chadha et al. (1979) also found that the results of the acaricidal treatments were not consistent. Bindra and Bakhetia (1971) also disproved bud-mite toxin theory for the following reasons. (1) They inoculated healthy mango plants with bud mites taken from malformed panicles. On inoculated plants large number of bud mite were noticed in the 3rd year of experiment but the inoculated plants remained as healthy as the unioculated ones. (2) The mother plants were sprayed with acaricide; when mother tree became free from mites, scion shoots were collected and grafted. The acaricides were regularly sprayed over the grafted plants to keep them mite free. Nevertheless, 56–67% incidence of malformation was recorded on the treated plants although the plants were free from mites. This shows that the disease is not due to simple feeding of mites. Lack of correlation between the bud mite population and incidence of malformation was reported by Wahba et al. (1976). They studied the occurrence of *Eriophyes mangiferae* and incidence of floral malformation in five varieties in Egypt. Although relationship between mango malformation and *A. mangiferae* populations was recorded on Mabrouka, Hindi and Taimour varieties, no such relationship was observed on other two (Zebda and Company). Recently, Freeman (2007) confirmed that the bud mite does not cause malformation as the *F. mangiferae* can cause the disease when mite is not present.

Mites as Vector However, Summanwar and Raychaudhuri (1968) revealed the role of the eriophyid mites in mango malformation as the vector. In 61% of the cases, colonies of *F. moniliforme* were obtained from mites collected from malformed portion trees and cultured directly on potato-dextrose-agar (PDA) whereas no fungal growth was obtained where mites were collected from malformed portions and were surface sterilized with mercuric chloride ($HgCl_2$) solution before culturing. Mites carry the fungus over its body and the injury caused by them

provides a means for the entry into host tissues. Sternlicht and Goldenberg (1976) recorded presence of *A. mangiferae* for the first time in Israel and did not find any direct role of these on inflorescence malformation. However, they did not exclude the possibility of its interaction with another biotic factor (a fungus). Pinkas and Gazit (1992) observed *A. mangiferae* as the transferring and wounding agent of *F. moniliforme* var. *subglutinans*. Similarly, Labuschangne et al. (1993) also recorded *A. mangiferae* to act as the vector of *F. moniliforme.* Recently, Freeman (2007) observed that spores of the pathogen did not attach to any particular part of the mite's body; but the mite was clearly capable of bearing these propagules. He also added that the pathogen is capable of penetrating the host without assistance from the mite. Chakrabarti et al. (1997b) for the first time observed presence of *Tyrolichus casei* Oudemans, in addition to *A. mangifera,* in large number of malformed as well as healthy panicles throughout the year. The interaction of *T. casei* with *F. moniliforme* var. *subglutinans* and mangiferin, the host defense metabolite, vis-à-vis over the manifestation of the disease symptoms were investigated (Fig. 5.3). *T. casei* and *F. moniliforme* var. *subglutinans* are positively correlated; but are affected adversely by mangiferin. At the initial stage of the disease development, a positive correlation was noted among the *Fusarium, T. casei* and mangiferin. *T. casei* carries large number of conidia and the fungal mycelia on the setae and body surface (Fig. 5.5a). When the mites were placed on PDA plate, furrow-like lines appeared over the media within 24 h due to movement of the mites (Fig. 5.5e). After 72 h of incubation, the growth of *F. moniliforme* var. *subglutinans* was observed over the furrows (Fig. 5.5b). The mites cause deep injuries on the bud surface, which

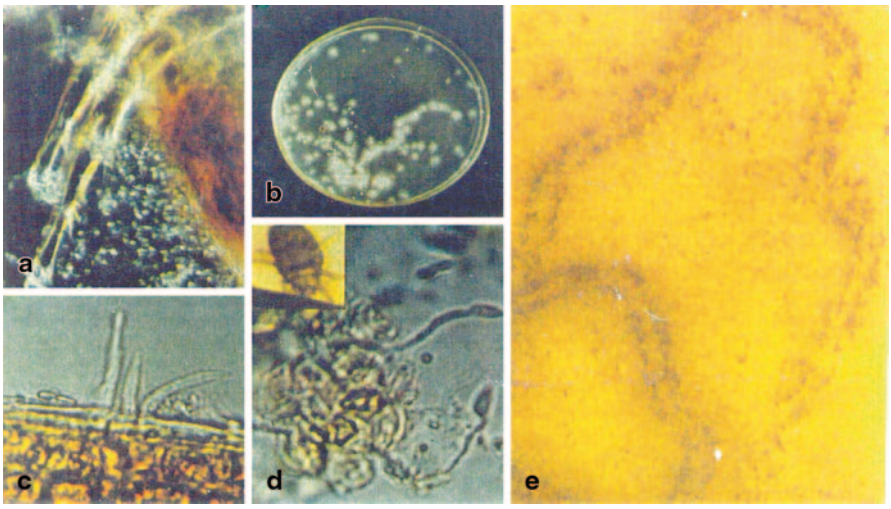

Fig. 5.5 Role of mite (*T. casei*) in mango malformation. Conidia of *F. moniliforme* adhered on the setae and body surface of *T. casei* (**a**), trail of the fungal colonies (**b**), formed along the pathway of movement of *T. casei* (**e**), ingress of the fungal hyphae deep into the host cells being prevented due to high accumulation of mangiferin in cortical cells (**c**), *T. casei* causing injury on the surface of mango buds (*inset*) and the infection peg from the germinated conidia reached inside the host cells through injuries (**d**)

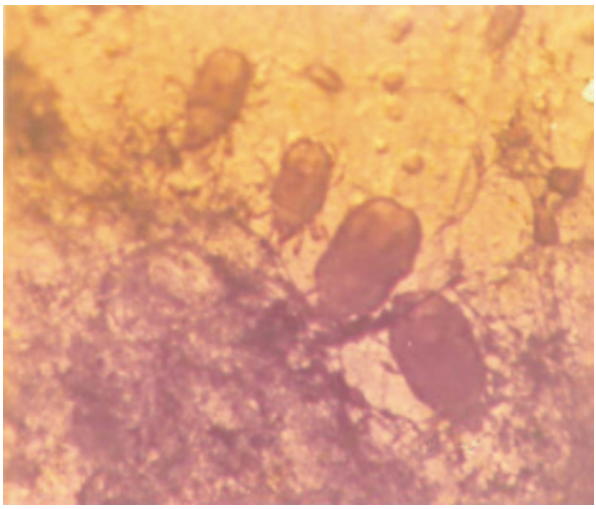

Fig. 5.6 *T. casei* feeding over mycelia of *F. moniliforme* var. *subglutinans*

allowed the fungus to enter inside the host (Fig. 5.5d). *T. casei* when was released over the fungus colony, the entire colony was found to be consumed by the mite (Fig. 5.6) within a week and a fast rate of multiplication of the mite was noticed. It seems that the mite (*T. casei*) visit the malformed panicles over surface of which *F. moniliforme* var. *subglutinans* grows abundantly, to feed on the fungus and in the process they disseminate the fungus to healthy buds. Gamliet-Atinsky et al. (2009a) observed that frequency and severity of infected buds were significantly higher in presence of mites, revealing their significant role in the fungal infection process. But they did not detect any wind borne bud mite bearing conidia in the trap and thus, concluded that *A. mangiferae* can carry and vector conidia between buds and assist in fungal penetration, but does not play a role in the aerial dissemination of conidia. These extensive researches reveal

- The mites are not able to cause mango malformation *per se.*
- They most probably help in vectoring the asexual infection propagules of the fungal pathogen, *F. moniliforme* var. *subglutinans.*
- This may best be the only means by which the fungal pathogen attacks the host.

Fungus

The morphological and cultural characters of the isolate of *F. moniliforme* var. *subglutinans* obtained from malformed mango tissues have been described by several authors. Varma et al. (1974) described the characters of the isolate No. IMI 152418 as follows: white mycelium appearing powdery due to microconidia; 0–1 septate, oval to fusiform microconidia from polyphialides; typical violet pigment; macroconidia lacking or rarely produced, 1–2 septate; chlamydospores lacking. Ploetz and Prakash (1997) also noted almost similar morphological features when the isolate was cultured on PDA. The mycelium are white, may be tinged purple, and

tan-orange; produces abundant oval-shaped microconidia which are usually single celled, but may have as many as three septa. Macro-conidia are fusiform or slightly sickle-shaped, and have a conspicuous, foot-shaped basal cell. Conidiophores are unbranched and branched polyphialides and monophialides; sporodochia are discrete or confluent. When sclerotia develop, they are often blue. Recently Britz et al. (2002) reported the distinguishing characters of the strain in more details. Colonies on PDA are with white, aerial mycelium, floccose (having tufts of soft woolly hairs). Reverse of colonies sometimes rosy buff (a medium to dark tan color) to dark purple. Microconidia variable in shape, obovoid (ovoid with the broad end toward the apex) conidia the most abundant type, oval to allantoids (shaped like a sausage) conidia occurring occasionally. Microconidia (Fig. 5.7b) mostly 0 septate: 4.3−9.0−14.4×1.7−2.4−3.3 µm. Macroconidia (Fig. 5.7c) long and slender,

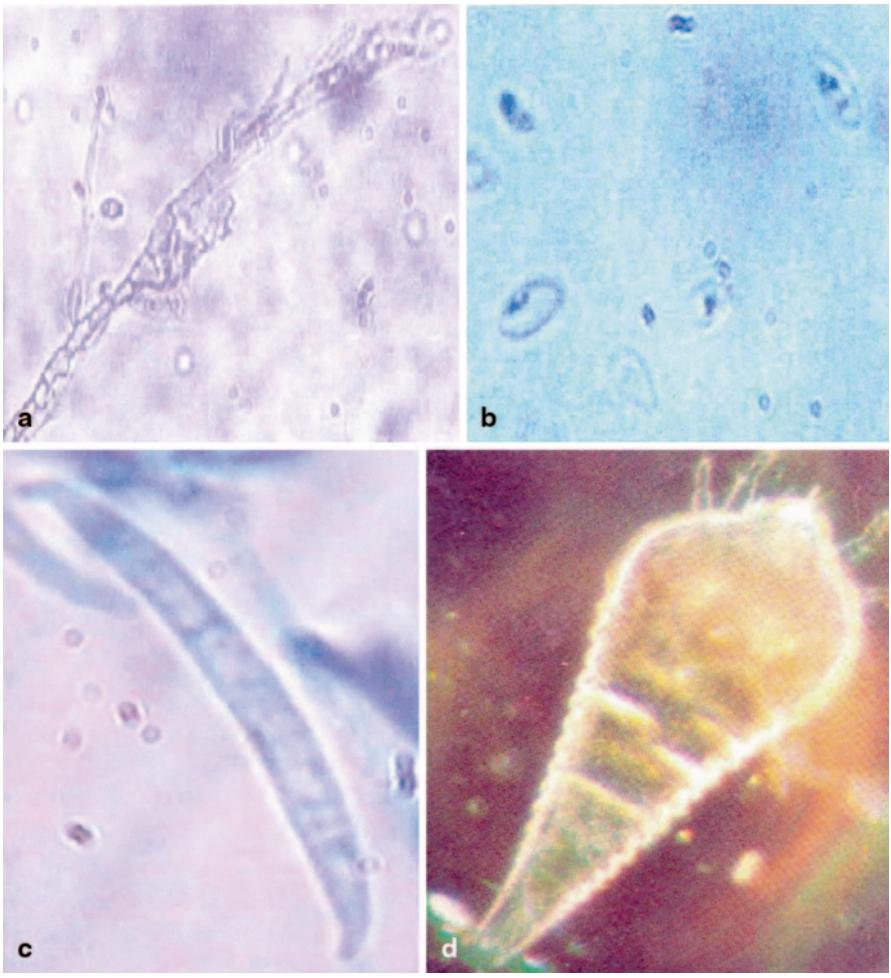

Fig. 5.7 Polyphialides (**a**), micro- (**b**) and macro conidia (**c**) of *F. miniliforme* var. *subglutinans* and the vector eriophyes mite (*Aceria mangiferae*) (**d**)

usually 3–5 septate: 43.1−51.8−61.4×1.9−2.3−3.4 µm. Conidiophores sympodially branched bearing mono-and polyphialides (Fig. 5.7a). Polyphialides have two–five condiogenous openings. Phialides on the aerial conidiophores monopolyphialidic, upto 30.0 µm wide. Sporodochia present, cream and orange. Chlamydospores absent. Sterile hyphae absent. Colonies on PDA with average growth rate of 3–4 mm/d at 25°C. Wollenweber and Reinking (1925) described macroconidia mostly 3-septate, whereas Booth (1971, 1977) described them to be 3–5 septate. Mitra and Lele (1981) in their study on cultural and morphological characters of the *Fusarium* made some interesting observations. They selected two isolates, one each from malformed vegetative (V) and malformed floral (F) tissues and one previously established but fresh pathogenic culture (P) from the type culture collection (Mycology Division, IARI) in their experiments. They observed that the size and shape of micro and macro-conidia produced by all the isolates were well within the range given by Wollenweber and Reinking (1925) and Booth (1971, 1977) for the taxon. Mitra and Lele (1981) recorded only one isolate, viz. (V) produced macroconidia which were all 2–3 septate. But size of the microconidia in (F) (8.2−11.8×2.6−2.9 µm) was bigger than that of (V) (7.8−10.7×2.1−3.1 µm). No chlamydospore or sclerotia were produced even in the very old cultures. Thus, they concluded that variations in morphology particularly in sporulation and pigmentation of the *Fusarium* as illustrated by different authors could possibly be due to the influence of the nature of host organs or the cultivar wherein the fungus was colonized. Recently, Freeman (2007) reported that PCR amplification of DNA using 1–3 F/R specific primer pair reaction was very reliable in identifying *F. mangiferae*.

Realignment of Nomenclature

The entry described by Snyder and Hansen (1957) as *F. moniliforme* now contains at least three different morphological species (*F. moniliforme, F. proliferatum,* and *F. subglutinans*). Nelson et al. (1983) used the following morphological characters to identify *F. subglutinans:* microconidia in false heads, but never in chains; microconidia produced on poly- and monophialides; falcate (curved like a sickle) macroconidia; no chlamydospores. Using this criteria at least six taxa possess the attributes of *F. subglutinans.* Several of the new species were previously named *F. subglutinans,* including one isolate from mango malformation pathogen. The study on mating types shows that the population of *G. fujikuroi* from mango is quite different from the population seen on mango. The vegetative compatible group (VCG) data from this group suggests that that there may be strains that have adapted specifically to mango (Leslie 1995). Steenkamp et al. (2000) examined phylogenetic relationships in the malformation pathogen with *β*-tubulin and histone H3 gene sequences. They indicated that a group of isolates from Florida, India, Israel and South Africa were closely related and were conspecific with isolates of '*F. subglutinans*' that had been previously shown to cause mango malformation worldwide.

Thus, Chakrabarti and Kumar (1998) proposed that the strain from *M. indica* might be considered as a special form within the species of *F. moniliforme* and be identified as *F. moniliforme* f. sp. *mangiferae*. Recently, these isolates were described as members of a new species, *F. mangiferae* Britz, Wingfield and Marasas *sp. nov.* (Britz et al. 2002).

In Vitro Culture of Fusarium moniliforme var. subglutinans

Chattopadhyay and Nandi (1975, 1981) investigated in details the *in vitro* growth and sporulation *of Fusarium moniliforme* var. *subglutinans* in presence of different carbon and nitrogen sources, vitamins, growth hormones and micronutrients. The salient features of their findings are as follows:

Carbon Sources Optimum growth was recorded on pectin followed in decreasing order by mannitol, starch, xylose and fructose. Sucrose, glucose and lactose were moderately used, and cellulose was very poorly utilized. In most cases, those sucrose sources supporting good growth also induced good sporulation.

Nitrogen Sources Maximum growth and sporulation were recorded on peptone. This was followed by aspartic acid and aspargine. Sodium nitrite among the inorganic nitrogen sources supported good growth and sporulation of the fungus. Growth and sporulation were moderate in potasasium nitrate, but poor in ammonium nitrate and ammonium sulphate.

Vitamin Sources Externally supplied pyridoxine and inositol supported no appreciable increase in mycelia growth. Biotin caused considerable growth; thiamine resulted in a fair amount of mycelia. Sporulation was also greatly increased by addition of vitamins except for pyridoxine which caused considerable reduction. Spore production was maximum at 0.01 ppm of biotin, but decreased gradually with an increase in concentration.

Growth Hormones All the growth hormones except indole acetic acid (IAA) stimulated growth. Maximum promotion became evident in maleic hydrazide (MH) followed in descending order of preference by gibberellic acid (GA) and indole butyric acid (IBA) and naphthalene acetic acid (NAA). MH was most effective in stimulating sporulation. All the hormones showed prominent inhibition at higher concentrations.

Trace Elements All the trace elements except molybdenum increased the mycelial growth at low concentrations. The mycelial growth was more than double at zinc (0.25 ppm). Iron, copper and manganese increased growth up to 0.25 ppm and then gradually decreased with higher concentration. Best sporulation was obtained in iron followed by zinc.

In another contemporary study on the topic the following observations were recorded (Mitra and Lele 1981).

Nutritional Studies

Carbon Maltose supported maximum growth followed by mannitol and sucrose. The best sporulation was noticed on starch followed by sorbitol, raffinose, glucose and fructose.

Nitrogen Among inorganic nitrogen salts and urea, good growth of the fungus was recorded on ammonium salts, best being ammonium phosphate indicating better utilization of ammoniacal nitrogen. Among nitrates, only magnesium nitrate supported moderately good growth. Among the amino acids tested, L-cystine promoted maximum growth. This is followed by L-glycine, glutamic acid, proline, aspartic acid serine and alanine in the receding order. Significantly poor growth was recorded by histidine, arginine and lysine. Tryptophan supported only moderate growth. Sporulation was generally less on amino acids. The fungus showed preference to organic form of nitrogen.

Vitamins Biotin promoted maximum growth. In addition, ascorbic acids, riboflavin and ca-pantothenate also supported good growth in receding order. Nicotinic acid supported fairly good growth. All the vitamins studied improved sporulation.

Trace Elements Boron (3 μg/ml) gave highest dry weight followed by zinc, copper, iron and manganese. Maximum sporulation was obtained at 0.5 μg/ml boron. Iron at 10 μg/ml showed toxic to the fungus and inhibited sporulation totally.

Hydrogen Ion Concentration The fungus grows and sporulates from pH 2–8. Maximum mycelial growth was obtained at pH 7.0 while best sporulation was observed at pH 6.0.

Temperature The fungus was grown over a temperature range of 10–40°C, of which 30°C was found best for growth and sporulation. The growth of the fungus was drastically reduced at 40°C.

Van Staden and Nicholson (1989) preferred to culture the fungus in Czapeck-Dox (CD) liquid medium under continuous light at 25 ± 2°C to induce cytokinin production by the fungus *in vitro*. But Kumar (1992) obtained maximum radial growth of the fungus in Richard's-agar medium over the PDA or CDA medium. Jourihar and Mehata (1973) found pH 4.0 as optimum for the growth of the fungus. It was later confirmed by Kumar (1992) when he reported that pH 4.4 was better than pH 7.4 in stimulating fungal growth. Kumar (1992) also observed the vegetative growth to increase with increasing incubation temperature from 20 to 25°C; but further increase in temperature to 30°C, affected/inhibited the growth. Singh et al. (1999) cultured *F. moniliforme* var. *subglutinans* on PDA at different temperatures (10, 12, 15 and 25°C). Growth of the fungus was minimal (0.39 mm^2) at 10°C after 10 days of incubation. The size of the colonies increased with increment of temperature and it reached the maximum (56.10 mm^2) at 25°C. Britz et al. (2002) cultured the fungus on carnation leaf-agar or KCL-agar at 23°C under fluorescent and cool white light with a 12 h photoperiod for conidia development while for pigment development the fungus was grown on PDA medium at 25°C in dark.

In general, *Fusarium moniliforme* var. *subglutinans* appears to have the ability to utilize a variety of C and N sources; thereby making it a very established organism in nature, capable of thriving under varied conditions of temperature and substrates, pH and exploiting vitamins and trace elements wherever available and hence, its elimination and control may prove to be problematic.

In vitro Germination of Conidia

Pandey et al. (2005) studied in detail the germination of conidia of *F. moniliforme* var. *subglutinans* both *in vitro* and *in vivo.* The conidia germinated within 5.30 h of incubation at 30°C. With further increase in temperature the conidial germination was adversely affected. At 40°C conidial germination was completely inhibited. At a temperature below 30°C, more time was required for germination of conidia. Increase in RH promoted the germination. The optimum RH was recorded as 90%. But further increase in RH delayed the germination. The conidia lost their viability gradually as the day proceeded from dawn towards dusk. The increase in concentration of conidia in the suspension affected the germination. Recently Krishnan et al. (2009) also reported that minimum time required to start germinayion was 6 h and maximum was recorded after 24 h.

In vivo Multiplication

The growth of fungus inside the host tissues is greatly influenced by the metabolites of the host while its growth and sporulation on the host surface is affected more by the environmental parameters (Chakrabarti et al. 1997; Babu-Koti and Rao 1998). Chakrabarti et al. (1997) recorded population density of *F. moniliforme* var. *subglutinans* on the host surface in different months of the year. In February, the fungal population was at its peak followed by a sharp decline in April–May. However, during July–September the inoculum density was pushed up again. But in the months of May and December–January, the fungal population was low. Babu-Koti and Rao (1998) investigated the frequency and intensity of the fungal growth inside the host tissues and observed profuse growth of the pathogen through intercellular spaces in shoot apices during March–April. In the subsequent summer months (May–June) apices are found without any mycelia pathogen. During monsoon, growth of the surviving mycelia in the buds restarted. During October–November the cells in apices again become severely colonized. Sporulation of the pathogen is not noticed in mango shoot buds. Chakrabarti and Ghosal (1989) observed that due to increase in concentration of mangiferin, the fungus is not able to proliferate on the malformed shoots and panicles. But there is concomitant oxidative transformation of mangiferin by polyphenoloxidase produced by the fungus resulting in the production of large amounts of polymeric quinones. The polymeric quinones do

not exhibit any perceptible anti-*Fusarium* activity. Thus as soon as accumulated mangiferin in these organs are transformed into polymeric quinones, the fungus grows abundantly over the surface of black lump of malformed panicles and shoots and sporulates. Varma et al. (1971) also reported that the fungus does not sporulate *in situ.* However, on drying malformed branches the fungus comes to the surface and sporulates. Noriega-Cantu et al. (1999) confirmed the sporulation by the fungus on the host surface when they trapped the fungal conidia by volumetric spore trapper in a malformed orchard. *F. moniliforme* var. *subglutinans* normally produces approximately 9,270 conidia/g plant material within 5 days under the prevailing ambient temperature of 35°C coupled with 90% RH (Pandey et al. 2005). For initial 3 days the conidia production takes place at an increasing rate, but thereafter the rate declines. Conidia production over dead panicles reached a peak within 81.9 h under favourable weather conditions. Under the climatic conditions of Mexico there are three peak periods of conidia production i.e. July, October–November and February (Noriega-Cantu et al. 1999). In Indian (eastern Uttar Pradesh) climates, two peak periods for the population of the *Fusarium* were noticed. The highest peak was in February. For the second highest peak, the population of the fungus started building up from June and finally attained the peak in August and then it waned (Pandey 2003). Gamliet-Atinsky (2009b) detected significantly higher number of conidia per gram of malformed inflorescence in May and June and then in April. They trapped higher number of conidia when RH values were less than 55%.

In vivo Germination of Conidia

Pandey et al. (2005) attempted to germinate conidia over the needle injuries at the base of the emerging buds. The germination of conidia over the host surface was poor although both temperature and RH were conducive for germination of the conidia. It was presumed that anti-fusarial host metabolite, mangiferin, might have oozed out through injuries and affected the germination. Natural wounds or injuries by mites or insects in nature were minute, which hardly stimulated mangiferin synthesis and accumulation in large amount resulting into more germination.

Host Invasion

The experimental evidences show that *F. moniliforme* var. *subglutinans* requires some exogenous wound and possibly a vector to facilitate host infection. The eriophyid mites, *Aceria mangiferae* (Sayed), are well known to cause the injury to the plants that provides a mechanism of the fungal pathogen into the tissues of the host plants. To reproduce the typical disease symptoms the sprouting buds were often inoculated with eriophyid or mycophagous (*Tyrolichus casei* Oudemans) mite (Chakrabarti et al. 1997). For example, usually, the mites that were carrying mycelia and conidia of the fungus on their body surface were collected from malformed

panicles and released over sprouting terminal buds (Nariani and Seth 1962; Summanwar 1967). In some experiments, the mites were collected from non-malformed plants and 3 days after their release over the terminal buds, a disk of the *Fusarium* was smeared at the base of the developing buds (Chakrabarti et al. 1997). These findings, indeed, demonstrate that feeding injuries are required for the fungal entry into the host. The injury to the host may not, however, be caused by mites alone. Other agencies, such as, lashing rains and hailstorms, birds, insects, human beings may also injure the plants and provide the fungus entry into the host tissue (Summanwar 1967). Usha et al. (1994) observed presence of much hair line cracks, pin sized to large holes and disorganized cells at the base of the buds in the cv. Amrapali. The bud meristametic region is probably the site of primary infection (Usha et al. 1994; Babu-Koti and Rao 1998). The fungal hyphae in form of filaments are found in close contact with the surface of leaf primordia. The site of infection is marked by accumulation of phenolic content in leaf primordial epidermis and mesophyll (Babu-Koti and Rao 1998). Recently the ability of *F. moniliforme* var. *subglutinans* to produce cell wall degrading enzymes (CWDE) both *in vitro* and *in vivo* were investigated (Kumar 2008). In the culture filtrates of the *Fusarium* pectin methyl esterase (PME) was absent, polygalcturonase (PG) was present in small quantity, and the amount of cellulae (C_x) was somewhat moderate; only the activity of protease was high (Fig. 5.8e, f). In malformed mango tissues also, protease showed the maximum activity while PME and PG (Fig. 5.8a, b) was moderate and C_x was very low (Fig. 5.8c, d). The above observations were further confirmed when Chattopadhyay and Nandi (1981) observed the fungus to utilize cellulose very poorly *in vitro* tests indicating low C_x activity of the fungus. The results thus suggest that *F. moniliforme* var. *subglutinans* has the potentiality to produce high amount of CWDE; but the host defense metabolites considerably inactivate the hydrolytic enzymes. Therefore, the role of cell wall degrading enzymes of *F. moniliforme* var. *subglutinans* in invading the host is very limited. However, Ibrahim et al. (1975) inferred that the fungus has the ability to penetrate the host cells mechanically.

Host Colonization

The mycelium was mostly present in cortex-phloem region and was intercellular. Occasionally in addition to intercellular mycelium intracellular mycelium was also seen (Varma et al. 1974; Ibrahim et al. 1975; Babu-Koti and Rao 1998). The invasion of hypahae in pith cells took place after the cells became less healthy (Ibrahim et al. 1975). Hyphae might be seen crossing the cells or lining their walls. Mycelial agglomeration was found in intercellular spaces (Varma et al. 1974) or within the cells, particularly in the pith cells (Ibrahim et al. 1975). Ibrahim et al. (1975) reported the discolouration of the xylem tissues and disappearance of sclerotic cells in the cortex layer of the diseased stem. The frequency and location of the mycelium was similar in floral and vegetative malformations. The plausible reason for localization of the pathogen mainly in the cortical region and its failure to reach the xylem vessels were put forward by Ghosal et al. (1979). Following the infection by

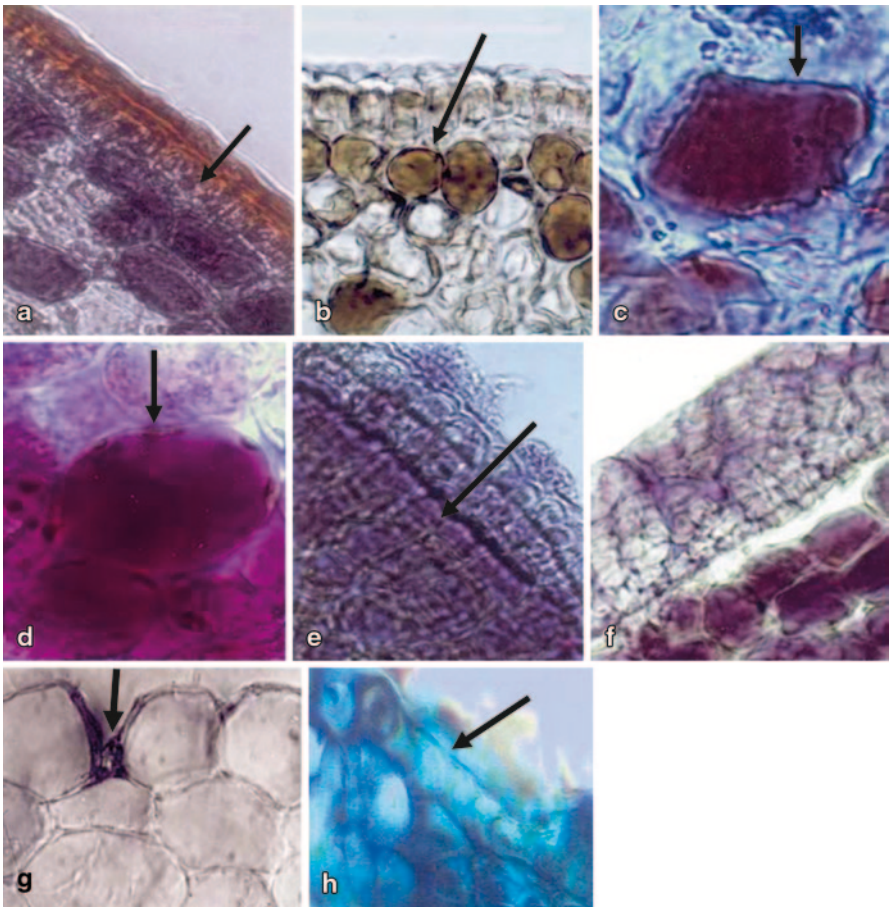

Fig. 5.8 Ferric chloride stained pectic substances in cell wall of healthy (*deep purple coloured*) (**a**) and malformed (*light coloured cells*, sporadically stained walls) (**b**) buds; safaranin stained cellulose content in cell wall of healthy (*thick red band*) (**c**) and malformed (*small coloured dots* over the cell wall) (**d**) buds; Schiff's reagent stained protein component in cell wall of healthy *coloured thick border*) (**e**) and malformed (no such border) (**f**) buds; enzyme induced gaps in between two cell (**g**); hyphae in the intercellular space (**h**)

F. moniliforme, in the infected mango buds the activity of *β*-1,3-glucanase increased by many folds (Kumar 2008). *β*-1,3-glucanase activity is known to induce phytoalexin, phenolics and pathogenesis related (PR) proteins (Wadhwa et al. 2001). In mango plants following the infection synthesis of a 20 KDa PR protein (Fig. 5.9) was reported (Chand and Chakrabarti 2003). When elicitors released by the pathogen comes in contact with the host receptor, the host responses by producing copius amount of *β*-1,3-glucanase which in turn degrades the glucan component from the hyphal walls of the *Fusarium* resulting into its lysis. Besides the host activates its defense genes that produce phenolics particularly mangiferin (Kumar and Chakrabarti 2010). The concentration of mangiferin in the fungal infected portion of the

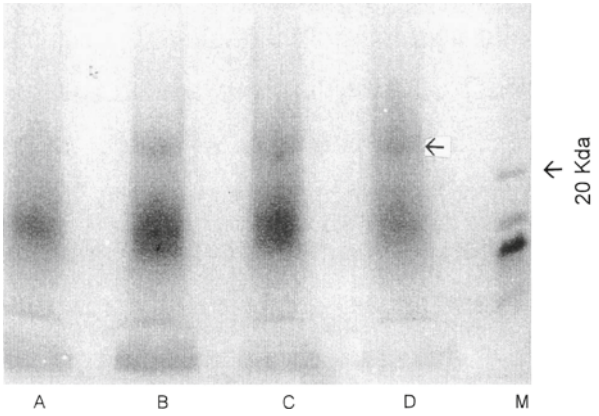

Fig. 5.9 SDS-PAGE of protein of mango buds. Marker protein (*M*), healthy (*D*), injured and inoculated (*C*), inoculated, injured and treated with hydrogen peroxide (*B*) and injured, inoculated and treated with cycloheximide (*A*)

apical buds was considerably increased (about 3–5 folds) over the control. The concentration of mangiferin was maximum in the cortical cells surrounding the fungus-infected ones. Its concentration gradually declined in areas away from the fungal infected zones. In the infected inflorescence also, the concentration of mangiferin was dramatically increased by about 10-folds over control within a period of about 4 weeks. The accumulated phenolic compounds in *Fusarium*-invaded cells inhibited significantly secretion and activity of CWDE (PG, PME and C_x) of the pathogen (Kumar 2008). However, the protease activity was not affected much (Fig. 5.10). The restricted activity of the CWDE can create only small gaps between the cells (Fig. 5.8g). Through these small gaps the pathogen can moves inside (Fig. 5.8h) the host to limited distance (Kumar and Chakrabarti 2010) but failed to go deep into the host cells and colonize extensively. Thus, the fungus remained localized at the

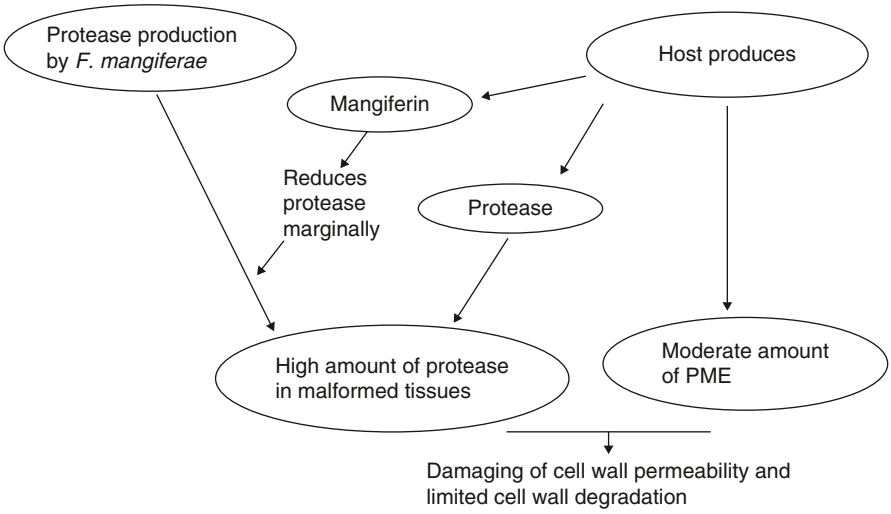

Fig. 5.10 Role of cell wall degrading enzymes in host penetration and colonization

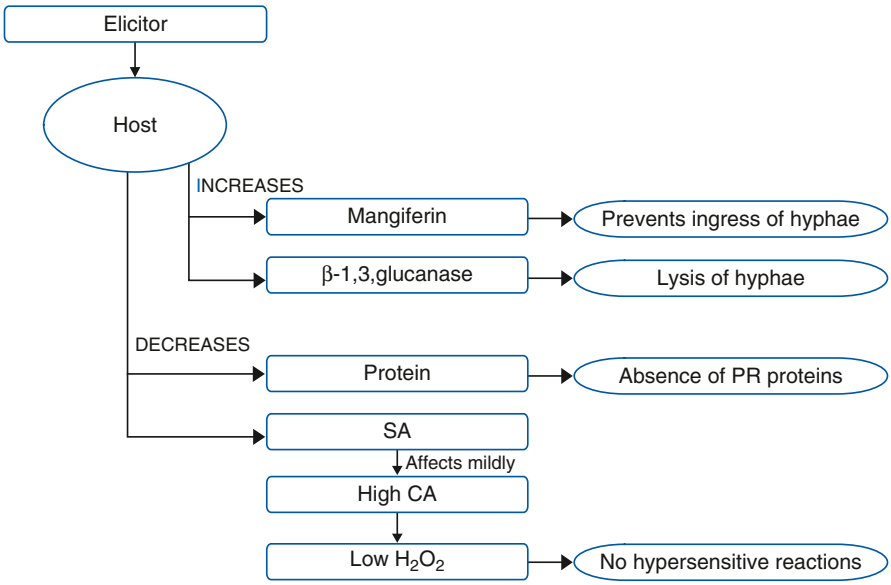

Fig. 5.11 Compatible interaction between *M. indica* cv. Amrapali and *F. moniliforme* var. *subglutinans*

outer cells of the affected parts. These observations are consistent with the reported localized nature (Summanwar 1967; Freeman 2007) of *F. moniliforme* infection of mango. Besides, over expression of *β*-1,3-glucanase in *Fusarium*-infected mango plants seems to cause lysis of *F. moniliforme* var. *subglutinans* in mango tissues, as was recorded in other fungal pathogens (Shetty 2002). Thus, *F. moniliforme* var. *subglutinans* although had the capacity to secret CWDE *in vitro,* but in the host cells *β*,1,3-glucanase-mediated mangiferin synthesis strictly restricted their activity permitting only limited host cell invasion and colonization vis-à-vis damage. Similar benign relationship between plants and certain facultative parasites with high potential to produce CWDE e.g. *Verticillium albo-atrum* was also observed by Cooper (1983). In a compatible host-pathogen interaction in mango (susceptible cv. Amrapali and *F. moniliforme* var. *subglutinans*), signal transduction system involving SA and H_2O_2 remain non-functional and defense chemicals are not synthesized. PR proteins, if produced, are significantly less in quantity. Defense genes that produce phenolics and *β*-1,3 glucanase become activated and save the plant from death but failed to stop symptom manifestations (Fig. 5.11) (Yadav et al. 2009).

Distribution of F. moniliforme var. subglutinans in Mango Panicles

Quantitative distribution of *F. subglutinans* over mango shoots and panicles was studied (Crookes and Rijkenberg 1985a; Darvas 1987; Ploetz 1994). They reported

the presence of *F. subglutinans* both from malformed and non malformed panicles. Unlike Crookes and Rijkenberg (1985b), Darvas (1987) and Ploetz (1994) observed the presence of the fungus only on branches that supported the malformed panicles, but never in tissues of branches over which non malformed panicles were developed. Levels of infection were highest in malformed flowers and vegetative shoots (65–85% of these tissues), were much lower or non-existent in asymptomatic tissues (0–11%) and rare in branches (0–4%) even when they supported malformed flowers or shoots. This prompted Ploetz (1994) to suggest that threshold a level of infection might be required before malformation symptoms developed on panicles. When within panicle infection was evaluated, an average of 84.5% of the small pedicel and peduncle tissue pieces from malformed panicle were infected. Ploetz (1994) recorded maximum population of the fungus at the base of the panicle and as the distance behind the base increased, the fungal population concomitantly decreased. Chand and Chakrabarti (2008) did not find presence of *Fusarium* inside non-malformed shoots and panicles and recorded *Fusarium* colonization in very high number at the basal (spring flush) and tip (autumn flush) tissues of malformed shoots. But it was absent in tissues in between (summer flush) (Fig. 5.12). These further confirmed that the pathogen does not move systemically. Maximum colonization of the fungus was observed at the tip portion of shoots and panicles (Fig. 5.13). It may be presumed that meristematic region is the primary site of infection causing malformation. It was interesting to note that the fungal population was more at nodal than at the internodal part. The fungus seems to get better footholding at the nodal region. Besides carbohydrate content at the nodal point was more. *F. moniliforme* var. *subglutinans* was absent inside tissues of healthy shoots and panicles. Nevertheless it was detected on their surface in good number. The pattern of distribution of *Fusarium* on surface of healthy shoots and panicles as well as its relative abundance inside the tissues of malformed ones showed similarity. Carbohydrate content in malformed panicles was more in comparison to the healthy ones. More was the number of the fungus in cells the greater was carbohydrate content. Similarly carbohydrate content of different parts of malformed shoots was more in comparison with their corresponding parts of healthy shoot. In malformed shoot quantity of total carbohydrate was less than that of the tips of healthy shoots. It is presumed that at initial stage of infection a metabolic sink was created at the site of infection and carbohydrate content became high. But after colonization of the cells extensively by the pathogen the carbohydrate content was reduced. Freeman (2007) recorded that in grafted plants, tissues in the region above the graft union to be more colonized than the tissues in the region below the graft and infection in seedlings descending from top to lower stem sections.

Host Specificity

The unpublished data of Varma et al. (1974) on the preliminary cross inoculation tests with strains of *F. moniliforme* indicate that the strain from mango alone can

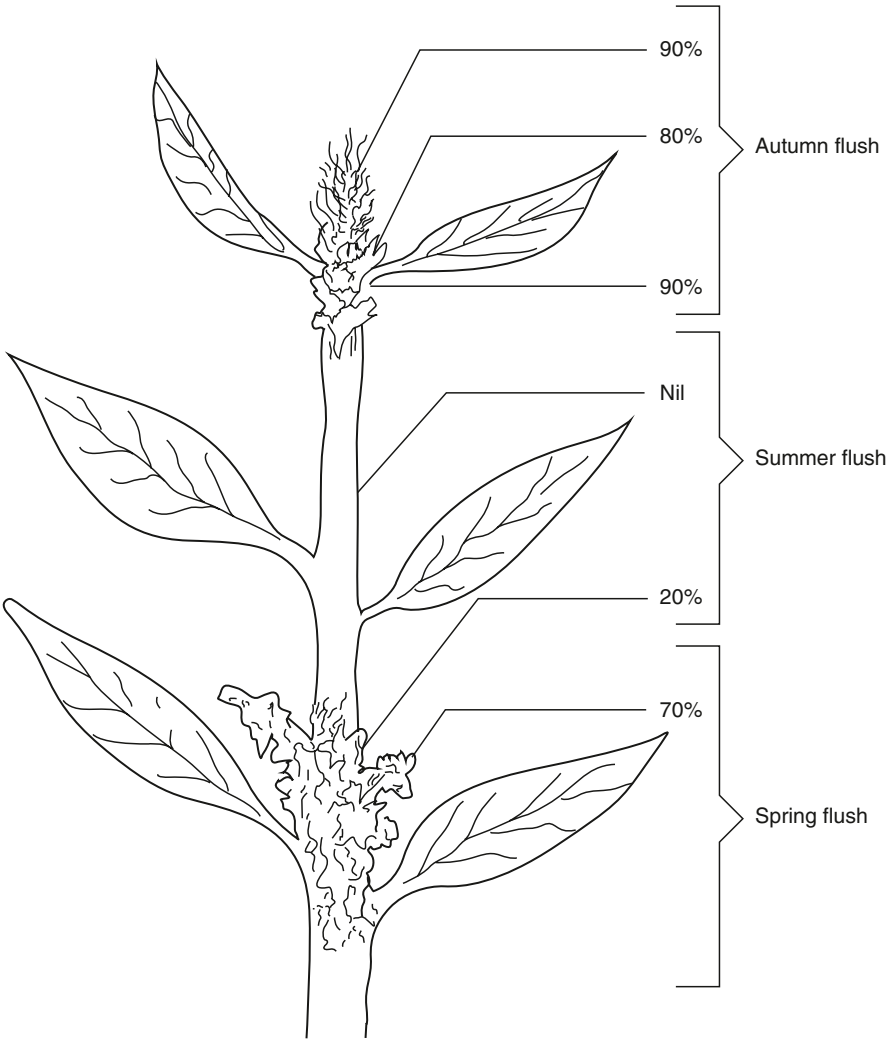

Fig. 5.12 Distribution of *F. moniliforme* var. *subglutinans* inside the cells of mango shoots

cause infection and symptoms typical of the disease produced i.e. it is host specific. Although seventeen different species were isolated from malformed vegetative and floral tissues but only certain isolates of *F. moniliforme* were found to be pathogenic and produced vegetative malformation (Ibrahim et al. 1975). The pathogenicity of other species of *Fusarium* in producing malformation symptoms was also tested (Salama et al. 1979). The spore suspensions of *F. oxysporum, F. solani* and an isolate of *F. moniliforme* from mango were injected into floral buds of 3-year-old healthy plants of mango cv. Hindy in Egypt in February. In the following March, abnormalities of terminal inflorescence developed when inoculated with *F. moniliforme* while other inoculated buds produced normal inflorescences. Freeman (2007)

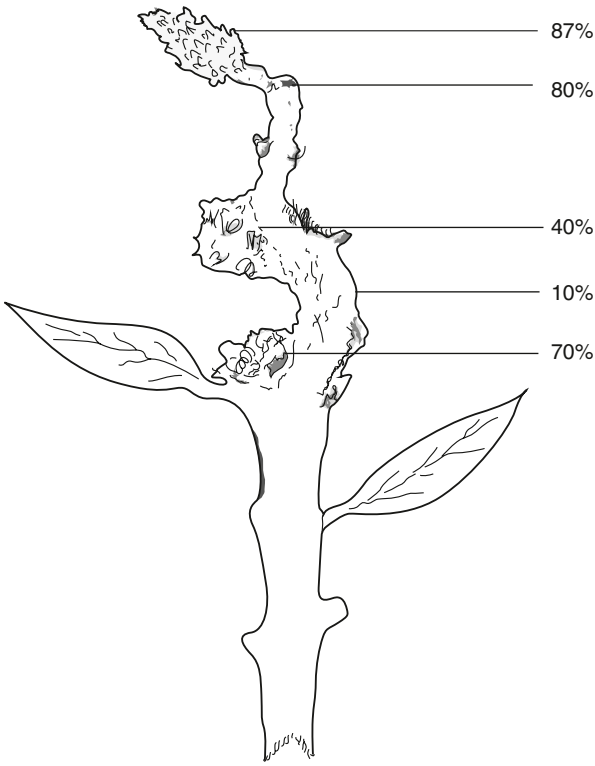

Fig. 5.13 Distribution of *F. moniliforme* var. *subglutinans* over mango panicle

reported that all inoculations with *F. oxysporum* did not yield the disease; only inoculation with *F. mangiferae* reproduced the disease symptoms.

The biochemical basis of host specificity of the isolates of *F. moniliforme* from malformed mango shoots and panicles was investigated (Kumar and Chakrabarti 1992). Three isolates of *F. moniliforme,* each one isolated from malformed mango shoot (IMI 225231), maize (IMI 204057) and banana fruits (IMI 225232) were compared for their biochemical response in elicitation of host defense system. *F. moniliforme* from the malformed shoots distinctly differed physiologically from the other two isolates. The isolates from mango shoots, unlike the other two isolates, did not produce fusaric acid *in vivo* and *in vitro.* Besides, in *F. moniliforme* from malformed tissues usually there was no pigmentation while other two isolates frequently produced pink and violet pigments. Its growth, in comparison to *F. moniliforme* from maize and banana, was more affected by higher temperature (>25°C) and pH (>6.5). The isolate from mango can utilize artificial growth media less efficiently.

Apical buds of *M. indica* cv. Baramasi of 5 year-old trees were inoculated with isolates from mango, maize and banana separately by the ‘slit inoculation technique’ (Summanwar et al. 1966). *M. indica* cv. Baramasi produced more mangiferin (defense metabolite of the host) in response to infection by *F. moniliforme* from

banana and maize. Thus, the production of high quantity of mangiferin indicates incompatibility between *F. moniliforme* from banana and maize with *M. indica.* All the shoots inoculated with isolates of banana and maize developed necrosis, became black and dried up to 3–5 cm behind from the point of inoculation. The attempts to reisolate the fungus from the inoculated shoot tips did not succeed. The hyphae in the host cells were killed by high content of mangiferin produced therein. But *F. moniliforme* from mango survived in the inoculated shoot tips owing to smaller amount of mangiferin and within 2 months after infection produced malformation symptoms.

Polyphenoloxidase (PPO) activity of the shoots inoculated with the isolates from maize and banana was higher than that of the mango. PPO degraded the mangiferin; more was the PPO activity, the lower was the mangiferin content. It is well known that mangiferin along with toxic metabolites of the *F. moniliforme* colonized in the host cells produced malformation symptoms (Chakrabarti and Ghosal 1989). Thus, in the mango buds inoculated with the isolates from maize and banana, due to massive and rapid degradation of mangiferin and eradication of *F. moniliforme* from the host cells, the malformation symptoms did not appear. But *F. moniliforme* from mango, due to its mild and slow action of PPO, degraded smaller amount of mangiferin. Hence, in the inoculated shoots, the lower rate of mangiferin production did not allow mangiferin content to attain cytotoxic levels while slow process of degradation never exhausted the stock completely and thus maintained same optimum concentration. Obviously, the biochemical events associated with elicitation, degradation and accumulation of mangiferin, the phytoalexin-like compound, determine the host specificity of *F. moniliforme* in *M. indica.*

Further investigations revealed the mechanism of development of a physiological race of *F. moniliforme* adapted specifically to mango (Kumar and Chakrabarti 1995). Subramaniam (1979) observed that varietal specificity of physiological races of a pathogen might be determined by the induction of metabolic changes in the host. *Fusarium* species react very readily to the substrate over which they grow by changing their morphological and physiological characters (Booth 1971). The host metabolites induce considerable changes in *F. oxysporum* growing over a susceptible host for an adequate period, developing it into a new physiologic race (Smith and Shaw 1943). The *Fusarium* has been reported to be present on asymptomatic plants and it requires prolonged period before development of malformation symptoms (Crookes and Rijkenberg 1985b; Ploetz 1994). It was presumed that the accumulated aberrant host metabolite mangiferin in the malformed tissues brings out the alteration of the fungus. To confirm the point, isolates of *F. moniliforme* from maize and banana were treated with mangiferin for about one year. Due to this prolonged mangiferin treatment, *F. moniliforme* isolates from maize and banana, like that from mango, lost the ability to use artificial medium efficiently, to grow profusely on banana or maize, and tend to produce hyaline instead of its normal rosy turned lilac coloured hyphae. Similarly effect of mangiferin on pigmentation of *F. moniliforme.* var. *subglutinans* from mango was also studied. White coloured hyphae (Fig. 5.14a) of *F. moniliforme* var. *subglutinans* produced pigmented hyphae (Fig. 5.14b) after repeated subculture without mangiferin. The pigmented hypahae (Fig. 5.14c)

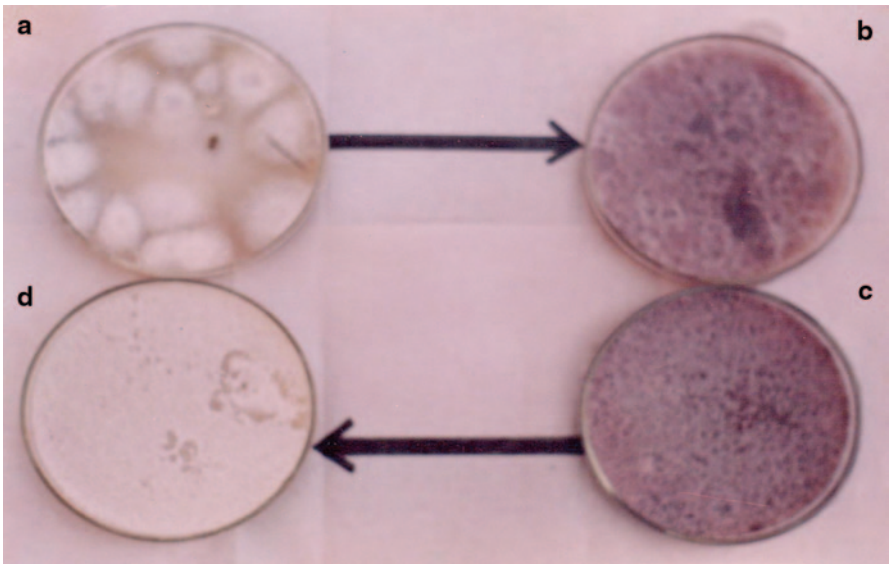

Fig. 5.14 Effect of mangiferin on pigmentation of *F. moniliforme*. *White coloured* hyphae (**a**) of *F. moniliforme* var. *subglutinans* produced *pigmented* hyphae (**b**) after repeated subculture without mangiferin. The *pigmented* hypahae (**c**) again turned white (**d**) after repeated subculture in presence of mangiferin at sub-optimal dose

again turned white (Fig. 5.14d) after repeated subculture in presence of mangiferin at sub-optimal dose. Mangiferin treatment increases cell pH (Chakrabarti and Kumar 1999). Higher pH enhances copper uptake but it has adverse effects on uptake of iron resulting in less pigmentation and low catabolic activity of the strains in the host cells (Cochrane 1958). The fusarial pigments are napthoquinone in nature and possess strong antimicrobial property that provides the fusaria an ecological advantage particularly when growing saprophytically. The less pigmented isolates may have some advantage in parasitism. The *F. moniliforme* isolate from mango in which both fusaric acid and pigment production was arrested, grew successfully as a parasite on mango. Mangiferin treatment increases C/N ratio of the hyphae of *F. moniliforme* var. *subglutinans* and high C/N ratio is known to help to establish the fungus as effective pathogen (Bollard and Matthews 1966). The absence of fusaric acid, that chelates zinc ion (Kumar et al. 1993) in mangiferin treated strains causes reduction of zinc content in the hyphal cells. However, in mango, the isolate after repeated subculture for one year partially regained its ability to produce pigments and to grow on artificial medium and host species other than *M. indica*.

Artificial Inoculation of the Host

Since the report of the association of *F. moniliforme* with malformed tissues, different inoculation techniques with the pathogen were tested to artificially reproduce

disease symptoms on the host. These methods of inoculation may be broadly categorized into five types.

Slit Inoculation The 8–10-month-old healthy mango seedlings were inoculated with the fungus (Summanwar et al. 1966) by cutting the growing points of all the seedlings were cut; (a) in the first set a longitudinal incision of ¾ to 1″ was made at the apical end of the seedlings. The culture was inserted in this incision and sterilized water-soaked absorbent cotton was placed over the cut ends and then were covered with polythene film to maintain moisture. The covers were removed after 3 weeks from the date of inoculations. (b) In the second set a sub-apical portion (2″ from the top) was inoculated after longitudinal injury. Wet absorbent cotton and polythene film were placed and removed as described above. After 1½ months of inoculation the malformed shoots appeared in the leaf axils on five out of seven and two out of three mango seedlings in the first and second experiments respectively. Reisolation from the induced malformation shoots yielded the same fungus. Thus, for the first time the Koch's postulates were established with the *F. moniliforme* isolate from mango. Varma et al. (1974) inoculated 15 plants (3 polyembryonic cv. Muvandan of 3 years old and 12 monoembryonic cv. Neelum of 6 months old) with *F. moniliforme* isolate from mango in January–February by the same vertical slit technique. After two months of inoculation one Muvandan plant and five Neelum plants developed floral malformation. But Prasad et al. (1972) after inoculating the twigs by this slit inoculation technique failed to reproduce malformation even after 8–9 months. In the seedlings also symptoms were produced only on four out of 20 inoculations but reisolation from these did not yield any fungus.

Kumar and Beniwal (1992) adapted the technique to reproduce the bunchy top symptoms. For this purpose they inoculated (a) the apex, (b) the axis of the uppermost leaf and (c) 15 cm below the apex. The only symptoms observed were shortening of internodes (in some cases), burning of leaf margins and shriveling of leaves. In seedlings where the apex was damaged due to inoculation, three or four branches developed. In some seedlings internodes remained shortened, thus clustering of leaves occurred. However, these symptoms did not resemble typical BT symptoms. Misra and Singh (1998) also reported that this technique has low percentage of reproducibility.

Inoculation by Injecting Spore Suspension Salama et al. (1979) first used this technique and its effectivity in reproducing the malformation was later confirmed by Freeman et al. (1999). Freeman et al. (1999) termed this technique wound inoculation. Salama et al. (1979) first surface sterilized healthy floral buds with 70% ethyl alcohol; then 0.1 ml of inoculum (conidial suspension) was injected into buds with a syringe in the month of February. A month later, abnormality of terminal inflorescence developed in four out of nine inoculated seedlings. Freeman et al. (1999) first transformed a wild-type isolate of *F. moniliforme* var. *subglutinans* from malformed tissues with GUS reporter genes, produced transformants with stable, single and multiple integration events. They tested the pathogenicity with the transformants and the wild isolates on the cultivar Kent. The conidial suspension (5×10^7 conidia/ml, 20 µl) was injected into dormant apical buds and maintained the

inoculated plants under green house conditions for 30 days at diurnal temperatures of 9–17°C for flower induction and thereafter at 17–22°C. Symptoms were observed 6–8 weeks after inoculation and no other organism except the GUS stained mycelia were found to be present in the malformed tissues.

Inoculation by Spraying Spore Suspension In this technique instead of making artificial injuries over the host surface, the advantages of presence of natural cracks caused due to temperature fluctuation or natural injuries inflicted by different agencies like mites, lashing rains and hailstorms, birds, insects, human beings (Summanwar 1967; Usha et al. 1994) have been availed to provide the fungus entry into the host tissue.

The malformation disease symptoms were produced in *M. indica* cv. Banarasi Langra by intentional infection as follows (Chakrabarti and Ghosal 1989). (a) A 3-year-old mango plant in a glass house was inoculated in December (2–3 months before flushing of new leaves) by spraying *F. moniliforme* var. *subglutinans* spore suspension (6×10^4 spores/ml) on/over apical portion of its shoots fortnightly for 3 consecutive months. High humidity was maintained. (b) Another 8–9-year-old mango plant, growing in a field, was inoculated by spraying with the *Fusarium* spore suspension on the apex of its one-year-old branches, every week, for 3 consecutive months. The first spraying was done in October (2–3 months prior to flowering), (c) Healthy mango inflorescence (15–25 cm long) was inoculated by spraying the spore suspension (50 ml). After inoculation, the inflorescence was covered with polythene bag; at the opening of the bag, a loosely set cotton ball was plugged to prevent the further ingress of any microorganism without restricting the inlet of air and moisture. The results of the three inoculation experiments were: (a) In March–April, the following year, the inoculated plants produced a few malformed shootlets at the apical portion, thereby exhibiting the bunchy top appearance. Subsequently, in July–August, most of the shootlets and leaflets dried up and profuse growth of pinkish hyphae of the fungus was observed both inside and outside of the host tissues. (b) In February–march, in the following year, most of the inoculated branches failed to set any flower. Only about 4% of them produced abnormal inflorescence. In June–July, the abnormal inflorescences dried up and were left to rot during the rain to give a black colour. Then, during October–November, pinkish mycelia of *F. moniliforme* var. *subglutinans* appeared on the surface of the black lump. (c) The florets of the inoculated inflorescence became necrotic, exuded profusely a syrupy liquid, withered and shed, leaving behind only the main axis on the twig. The bare panicles finally got detached and malformed shoots emerged from the point of detachment. Ploetz and Gregory (1992) also reproduced both stages of the disease by spraying spore suspension. Freeman et al. (1999) achieved success in symptom production by placing a conidial drop on dormant buds without wounding, which further demonstrates the virulence of the pathogen.

Bud Mite Mediated Inoculation This is another simple technique to reproduce the malformation symptoms in nature's way. Puttarudriah and Channa-Basavanna (1961) took the eriophyid mites from malformed twigs and introduced them to just sprouting leaf buds at the terminal region of healthy seedlings. The inoculated buds

at the end of 3 months produced malformed symptoms. In a separate experiment, about one year old seedlings were inoculated with the mites collected from the diseased mango plants in the following manner (Nariani and Seth 1962). More than 10 living eriophyid mites were picked up and pinned in the leaf scales of the terminal buds or in the axillary buds of healthy plants. Moist cotton was wrapped just below the inoculated point to provide high humidity. The plants were covered with bell jars. Within one month of incubation proliferation of buds were noticed and typical malformed bunch was formed about 3–5 months after. The disease could be induced during two active growth periods of the mango plants i.e. March–April and July–October. Similarly, Labuschangne et al. (1993) reproduced the disease symptoms by inoculating the apical buds with bud mites carrying the *Fusarium* on its body surface. But Bindra and Bakhetia (1971) experienced this technique as ineffective in inducing malformation. They picked up fifty bud mites from malformed shoots and placed them on the terminal buds of the test plants in the month of February. The plant terminals were then covered with polythene bags for the next 5 days to maintain high humidity. But no malformation symptoms were developed as reported by the earlier workers.

Inoculation After Suppressing Host Defense System It has been observed that mangiferin, the defense metabolite of the host plant, accumulates in large amounts in and around the invaded host cells and thus prevents the pathogen to be established. Mangiferin also accumulates in response to other injuries at the inflicted sites of the host. The deeper is the injury, the more is mangiferin accumulation vis-à-vis host resistance (Chakrabarti and Ghosal 1989). As *F. moniliforme* var. *subglutinans* is unable to enter the host without any wound, to develop an appropriate inoculation technique to reproduce malformation symptoms proved to be a tough challenge to the pathologists. Consequently, lack of inoculation technique that yields consistent results has been detrimental to confirm the etiology and development of control strategy. It was presumed that arrest of elicitation of host hypersensitive reaction (HR) in the form of accumulation of mangiferin by a translation inhibitor, cycloheximide or conversion of mangiferin into non-efficacious form i.e. polymeric quinone with the help of an oxidant (hydrogen peroxide) at the initial stage of infection might help the pathogen to be established in the host cells and subsequently to produce toxins and thus the disease symptoms. In view of above information, Chand and Chakrabarti (2003) inflicted micro needle injuries over the floral buds of cv. Amrapali during inception stage (November) and inoculated with the inoculum grown on the host tissues (inoculum strips). Prior to inoculation, buds were treated with hydrogen peroxide solution (2%) and after inoculation, cycloheximide solution (2 ppm) was sprayed over the inoculated buds. Hydrogen peroxide detoxified mangiferin (the host defense anti-fusarial compound) while cycloheximide affected its biosynthesis. Inoculated buds produced 60–67.5% malformed panicles in next March and 60–70% vegetative shoots in November. The disease develop when the temperature was mild (8–19°C) and RH was high (>85%). Later Chand and Chakrabarti (2004) attempted to reproduce symptoms of vegetative malformation based on the above principles. The experiment was conducted on a 7-year-old

plant of cv. of Amrapali during July–September. The apical buds, after micro needle injuries at the base were sprayed with cycloheximide (2 ppm) and then inoculated with the inoculum strips. Of the treated buds, 33.35 turned malformed, 35.3% buds remained in quiescent condition while the rest 31.4% became healthy shoots.

Induction of Malformation in Mango Buds

The mechanism by which the mango buds are induced into malformation has long been an intriguing question. The sequences of development of the floral buds in mango are divided into five developmental stages (Hifny et al. 1978). The first stage was at bud burst (Fig. 4.3) when buds either containing healthy or malformed inflorescences simultaneously burst. Both develop nearly at the same time. However, even at this stage it is possible to differentiate between healthy and malformed buds. Malformed buds are more compact than healthy ones and contain excessive scales and undeveloped leaves at their base (Fig. 4.3b, c). In the subsequent stages, the symptoms assume typical form. Similarly, Singh and Dhillon (1990b) divided flower development process into four stages. They designated the fully swollen apical bud stage as the first of the developmental stages. The length (2.5 cm) and breadth (2.7 cm) of malformed fully swollen buds were significantly greater than the length (2.1 cm) and breadth (0.9 cm) of the healthy ones. Thus, both types of buds from the very inception are quite distinguishable by their dimensions. The morphological observations suggest that the actual beginning of the floral malformation occurs in buds long time (5–6 weeks) before bursting (Hifny et al. 1978).

The transition from healthy to malformed growth is also associated with various biochemical changes within the host (Hifny et al. 1978; Singh 1986; Babu-Koti and Rao 1998). For example, some metabolites of the host viz. non-reducing sugar, total sugars, total phenolics and proteins accumulate in considerably higher quantity in the fully swollen apical buds destined to be malformed. On the other hand, constituents like DNA, RNA and total free amino acids, essential for normal growth, reduce below the normal level at this stage. Further it has been observed (Chakrabarti and Sharma 1993) that leaves from axils of which buds for malformed panicles and shoots are formed, contain more mangiferin than the leaves attached to the healthy flowers buds. During the time of bud initiation, there is an influx of mangiferin from leaves to the attached buds. Thus, the buds in which mangiferin content reaches the supraoptimal level grow abnormally into malformed panicles or shoots. A gradual augmentation of phenolic accumulation is the resultant effects of pathogen interaction (Babu-Koti and Rao 1998). Mangiferin has also been found to accumulate in high amounts in the cells surrounding the pathogen-invaded ones following infection (Ghosal et al. 1979). For continuously three months i.e. July–September usually the fungal population over the host surface is counted very high (Chakrabarti et al. 1997a). In nature peak period of production of conidia, the infectious entities, is July–August. In October–November, the cells of shoot apices are found to be extensively colonized (Babu-Koti and Rao 1998). Therefore, the

conidia produced during rainy season initiate fresh infection on shoot apices; the host tissues are colonized in adequate proportion with concomitant serious biochemical alterations in the infected tissues (Chakrabarti et al. 1990) by October prior to the flower bud differentiation for the next flowering season. Such buds heavily colonized by the fungus and those containing high amounts of the fungal toxins and aberrant host metabolites are transformed into abnormal inflorescences or malformed shoots.

Other Fungal Species Associated with the Malformation

Bhatnagar and Beniwal (1977) and Kumar (1983) isolated *F. oxysporum* from bunchy top affected mango seedlings. They observed, on inoculation of healthy seedlings through soil, development of some of the symptoms of vegetative malformation, such as, stunting of growth, shortening of internodes, scaly leaves and hypertrophied growth at the seedling apex. But the symptoms appeared after more than one year. Recently, Freeman (2007) recorded that all inoculations (through soil and aerial parts of the seedlings) with *F. oxysporum* failed to yield the disease. Thus, the report that *F. oxysporum* caused mango malformation has not been corroborated. According to Ploetz and Prakash (1997) this might be due to the misidentification of *F. subglutinans* because on PDA, *F. subglutinans* resembles and may be confused with *F. oxysporum* (Nelson et al. 1983).

Britz et al. (2002) found that in addition to the species *F. mangiferae, F. sterilihyphosum* Britz, Marasas & Wingfield, sp. nov. and one undescribed species of *Fusarium* were also associated with malformed tissues. *F. sterilihyphosum* has only been isolated from malformed mango tissues in South Africa. This species is morphologically similar to *F. mangiferae. F. sterilihyphosum* has shorter 3–5 septate macroconidia, faster growth rate on PDA at 25°C than *F. mangiferae* and produces sterile coiled hyphae. All the three isolates of the undescribed *Fusarium species* were isolated from mango tissues in Malaysia. The species differed from *F. mangiferae and F. sterilihyphosum* by having conidiogenous cells with more than three openings and relatively short 3–5 septate macroconidia. However, it is not known whether *F. sterilihyphosum* or the undescribed *Fusarium species* are able to cause the disease on mango trees. Recently Zhan et al. (2010), Lu et al. (2010) and Yanchao et al. (2010) reported the association of *F. proliferatum* (Matsushima) Nirenberg with both vegetative and floral malformation and induction of malformation on mango seedlings by artificial inoculation with the fungus. According to Rodriguez-Alvarado et al. (2010) there are at least 9 phylogenetically distinct fusaria within the *Gibberella fujikuroi* sp. complex associated with malformation worldwide. These include one sp. with the African clad (*F. pseudocircinatum*), two species with Asian clad (*F. mangiferae* and *F. proliferatum*) six species with American clad (*F. sterilihyphosum* and five undescribed). One of the undescribed species is most common in Mexico (*Fusaium* sp. nov.ex *Mangifera indica* L).

During a recent survey by Khaskheli et al. (2008) in Sindh of Pakistan, six fungal species viz., *Fusarium nivale* (Fr.) Ces, *F. oxysporum, F. moniliforme, F. semitectum, Alternaria alternate* and *Aspergillus niger* were isolated from malformed mango tissues. *F. nivale* was predominantly isolated from malformed inflorescence.

Noriega-Cantu et al. (1999) also isolated other fungal genera like *Pestalotia, Botryodiplodia* and *Aspergillus* but at low frequencies.

Ibrahim et al. (1975) isolated the following fungi from undifferentiated buds, vegetative and floral malformations: *F. moniliforme, Fusarium species, Botryodiplodia theobromae, Nigrospora* sp., *Helminthosporium* sp., *Curvularia* sp., *Verticillium* sp., *Botrytis* sp., *Cladosporium* sp., *Geotrichum* sp., *Pestalotia* sp., *Stemphy lium* sp., *Epicoccum* sp., *Monocillium* sp., *Sporobolmyces* sp., *and Merothecium* sp.

The role (if any) of the above mentioned isolates in manifestation of malformation have not been investigated or confirmed. Most likely they belong to the phyllosphere of mango or at the best these are secondary organisms thriving on dead necrotic malformed shoots and panicles.

Chapter 6
Epidemiology

Various climate, weather and host related parameters affect the tempo of disease development and progression. The major ones on which significant data have accumulated are discussed.

Host Age

It appears from various reports that host age is linked with the types of malformation and disease severity. Singh and Chakravarti (1935) found the disease to be confined to younger mango trees. All the plants suffering from cent percent infection were at very young age. Nirvan (1953) observed that the initial symptoms of the disease appear when the plants are hardly 3–4 years old. The symptoms on these plants are bunchy top. Singh et al. (1961) recorded the incidence of both vegetative and floral malformation on plants belonging to different age groups. The malformation on 5 months, 9 months, one and half year and two years old seedlings were 0.8, 18.4, 25 and cent percent respectively. In the trees between 4 and 8 years age group, the incidence was recorded on 90.9% of plants. The intensity of vegetative malformation on trees of this age group varied from 2.7 to 80.4%. However, with increasing age, the plants suffer less from vegetative malformation. Trees of 25 years or more in age show very few shoots affected with vegetative malformation. But incidence of floral malformation is recorded in ascending order with advancing host age. Thus, 5 year-old plants that had been suffering from 21.9% floral malformation in 1954, were found to be inflicted with 29.5% floral malformation only after 4 years (Singh et al. 1961). The incidence of floral malformation on trees of 15 years age varied from 32.12 to 92% in Uttar Pradesh. The affected trees showed variation of malformed inflorescences on individual trees from 2 to 80%. Hence, the intensity of vegetative malformation is maximum in mango trees aged below 10 years and there is a subsequent decline in the intensity of vegetative malformation as the age of trees increases. But seedling mango plants show minimum intensity of floral malformation (Mallik 1963). Overall incidence of malformation is greater on young than in old plants (Puttarudriah and

D. K. Chakrabarti, *Mango Malformation,*
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Channa-Basavanna 1961; Singh et al. 1961). A survey was conducted (Chadha et al. 1979) to find out the extent of malformation in trees of different age groups (0–5, 5–10, 10–15 and 15 years and above) in the same variety. The highest incidence of malformed inflorescences was noticed in the age groups of 5–10 years. Young trees of just bearing stage showed the highest incidence. The percent incidence of malformation decreased with increase in age.

Puttoo et al. (1975) surveyed the disease incidence among 13 commercially important mango cultivars. He recorded the disease incidence ranged from 2.8% in young trees to 100% in trees over 10 years old. Ram et al. (1990) found trees of age of 6–15 years were more highly susceptible than older trees of 16–26 years. Sharma and Badiyala (1990) observed that disease incidence was highest in trees less than 10 years old, decreased with increasing age irrespective of cultivar. Pandey et al. (2003) reported that incidence of vegetative malformation in Dashehari was maximum when plants were 5-year-old. The disease incidence decreased from 5 to 20-year-old trees with an average rate 10.82% per annum. The optimum host age for maximum intensity of floral malformation in Dashehari was found to be over 11 years. Decreasing incidence of both vegetative and floral malformation with age seems to be affected by the vigour and biochemical components of trees (Pandey 2003). In aging plants production of panicles and shoots are reduced gradually resulting into decrease in number of infection sites vis-à-vis development of malformed shoots and panicles.

Not only the age of the tree, even the the age of the bearing shoots is correlated with the disease incidence (Shawky et al. 1980). The disease incidence increases as the bearing shoots become aged. Thus, the highest percentage of disorder is associated with the spring flush, decreases in the summer flush and reaches a minimum in the autumn flush. The above phenomenon seems to be due to the fact that the early autumn shoots are too young to accumulate sufficient metabolites required for flowering; hence the number of flower buds, the site of infection, is in lesser number. Shoots of summer and spring flushes get plenty of time to accumulate metabolites required for flowering. But vegetative buds developed during summer flush usually escape infection due to inclement weather condition vis-à-vis low fungal population while those developed during the spring flush get infected in higher proportion as the population of the pathogen is very high during this period and the prevailing weather parameters are highly conducive for infection (Chakrabarti et al. 1997a).

Bearing Habit

Most of the north Indian popular mango varieties are of irregular bearing habit. They either flower erratically or at one year interval which is known as the alternate bearing phenomenon. To overcome this problem some mango varieties like Neelum have been introduced into the northern belt. Besides, new hybrids like Amrapali and

Mallika have been developed to overcome the problem of "off year." Unfortunately, Mallika and Neelum are highly susceptible to malformation (Kumar and Beniwal 1992). Neelum which is a regular bearer in southern India is severely affected by both "off year" phenomenon and malformation in the north (Mallik 1963). Similarly, the incidence of malformation is very high in Amrapali (50%) and Mallika (55%) (Badiyala and Lakhanpal 1990). Whereas the alternate bearing cultivars like Dashehari (Singh and Jawanda 1961; Sharma 1953), Langra (Jagirdar and Shaik 1968) have been consistently found to be less susceptible. A low disease percentage has been recorded in Langra (4.37%) (Badiyala and Lakhanpal 1990) and Chausa (10.82–24.2%) (Ram et al. 1990). In years when the trees bloom profusely, the intensity of malformed panicles is greater but in "off year" when there is less number of inflorescences, the incidence of floral malformation is also less (Singh et al. 1961). It has been recorded that during "off year" because the flowering is very less, the production of malformed panicles declines (Kumar and Chakrabarti 1997b). Thus, during "on year" there is a lack of initial inoculum at threshold level; consequently there is lesser infection (Chakrabarti et al. 2005). On the other hand, in regular bearing cultivars, there is steady build up of inoculum and in every year sufficient fusarial population are available to initiate the disease in the next season.

Time of Flowering

Among various factors that influence the occurrence and severity of the disease, time of flower bud initiation plays an important role. A noticeable trend observed is that most of the mid and late season varieties do not show the disease or they had only few affected inflorescence (Singh et al. 1961; Chadha et al. 1979). In the cultivar Neelum during the flowering of February–March, 60% malformed panicles were recorded whereas the same tree had only 4.5% malformation during off-season flowering in June (Majumder and Sinha 1972). Singh et al. (1979) recorded the incidence of floral malformation on four cultivars in consecutive two years. Under north Indian conditions in the cultivar Dashehari, the flower bud burst starts at the end of January and continues up to the end of February. The buds that emerged early in the season showed floral malformation up to 26.9%. But the buds in the same tree that emerged one month after, suffered only 3.4% infection. They inferred that early emerging flower buds were severely affected whereas later emerging ones escaped the disease. Khurana and Gupta (1973) also made similar observations. On the contrary, Kulkarni (1979) observed that malformed panicles emerged at later months (February–March) than the normal ones (December–January). He recorded high incidence of malformation on the cvs. Thambu and Gurd, although both are late season cultivars under Andhra conditions. Hence, he concluded that there exists no correlation between time of flowering and susceptibility to malformation. Obviously, more experimental evidence is needed in support of such a generalization (Kumar and Beniwal 1992).

Effects of Environmental Factors on the Disease Development

Temperature

The malformation in mango appears in severe form in North-West region which is thought to be due the severe cold in January–February (average temperature 10–15°C), prior to flowering (Jagirdar and Shaik 1968). It has also been pointed out that the disease is mild in the areas where temperatures lie from 15 to 25°C, and sporadic where the same was 20–25°C. High incidence of the disease has been recorded mainly from areas where mean winter temperature is less than 16°C (Varma 1983). The growth of the fungus *in vitro* is inhibited approximately at 35°C and beyond 55°C, the fungus becomes totally inactivated both *in vivo* and *in vitro;* consequently, the summer growth of plants escapes infection (Varma et al. 1971). The high disease incidence in Neelum during its flowering in February–March and a significant reduction in development of malformation on the flowers developed in June were correlated with prevailing temperature at the time of flowering (Majumder and Sinha 1972). This was confirmed by artificially raising the temperature around Neelum trees during February–March which resulted in reduction in malformation (Majumder and Sinha 1972). Thus, it was conjectured that the sporadic incidence of the disease in the South might be due to constantly high temperature and lack of cold spell like that of the north. Chadha et al. (1979) also considered that there is a negative correlation between prevailing temperature conditions during the period of panicle emergence and incidence of malformation. Singh et al. (1979) attributed lesser incidence of the disease in late season varieties to relatively higher temperature during panicle development, particularly before the balloon stage. For example, in cv. Dashehari, when the panicles emerged on January 21, the maximum and minimum prevailing temperatures were 23.7 and 6.9°C. respectively; concomitantly the disease incidence was very high. But in the same plants, when flowers developed in February under higher prevailing minimum (10.5°C) and maximum (27.7°C) temperatures, the incidence reduced to a minimum level. Singh et al. (1999) surveyed the incidence of floral malformation of mango in Kumaon hills of Uttara Khand, India up to 1,700 m altitude and related the level of disease incidence with prevailing temperature. The floral malformation was maximum (20%) at an altitude of 400 m at Kathgodam while it was almost nil at an altitude of 1,250 m and above. The night temperature below 10°C for long periods (16–18 h) at higher altitude seems to be responsible for suppressing the incidence of malformation. This was corroborated with laboratory findings that the fungal growth was inhibited below 10°C under controlled conditions. To confirm the adverse effects of high temperature on disease manifestation, the ambient temperature around mango trees cv. Amrapali was elevated by covering it with polythene sheet during flowering (Singh et al. 1998). The polythene cover increased the average maximum and minimum temperatures by 4.1, 0.9°C and RH by 4% respectively. Thus, higher temperature coupled with high humidity inside the polythene cover adversely affected malformation (Tripathi and Ram 1995). It is well

known that moist heat is more effective on biological system than dry heat. Based on such observations, it has been extrapolated that relatively higher temperature and humidity possibly lead to rare occurrence of malformation in southern part of India. Noriega-Cantu et al. (1999) observed that the association between disease incidence and climatic variables reflected a strong dependence of disease development on microclimatic factors measured at the canopy level. The cumulative disease incidence did not increase when the maximum daily temperatures and the average temperature per hour increased and prevailed at levels greater than 33 and 25°C respectively, usually from March to May. The nurseries under shade and inside malformed mango orchards had higher disease incidence while less in poly house at 25–31°C (Gaur and Chakrabarti 2009). Higher temperature, in addition to the direct inhibitory effect on multiplication of the fungus, increases mangiferin production *in vivo* and high level of mangiferin content in turn adversely affect the fungal population (Chakrabarti and Ghosal 1985).

Relative Humidity (RH)

The occurrence of greater incidence of malformation in sub-mountainous districts of Punjab than in drier areas in plains pointed towards the possible role of RH in development of malformation (Singh and Jawanda 1961). Noriega-Cantu et al. (1999) recorded highest spore density during the rainy season, when humidity (92–94%) was relatively high.

In addition to temperature and RH, sunshine is also an important environmental parameter. In nature, viability of conidia of *F. moniliforme* var. *subglutinans* was maximum in the early morning hours and there was a gradual loss in viability as the day proceeded from dawn to dusk (Pandey et al. 2005). High temperature and light intensity adversely affect viability. Rotem (1988) also reported that survival of conidia of *Fusaruium* species was strongly affected by sunlight. Gaur and Chakrabarti (2009) reported that the disease incidence showed significant positive correlation with total rainfall, negative with higher temperature, positive but non-significant with RH.

But under natural conditions, effects of different parameters like temperature, RH, the fungal density and host metabolites (read mangiferin for malformation disease) are overlapping. Therefore, response of disease progress might not be linear function of environmental parameters under field conditions (Shaner 1981). Seasonal variation in the incidence of malformation is the joint function of temperature, RH, the fungal density, vector population, the host metabolites particularly the amount of host defense metabolite, mangiferin.

Multiplication of the fungus was enhanced by high RH (Chakrabarti et al. 1997a). During the spring flush (February) range of temperature was mild (8–27°C), mangiferin content was low (5.75%) and average RH was high (84.8%) resulting in maximization of population of the pathogen (228 c.f.u g^{-1} plant material) vis-à-vis disease incidence. The maximum density of *F. moniliforme* var. *subglutinans* on

mango shoots at moderate temperature was recorded by Nath et al. (1987) (12–27°C) and Noriega-Cantu et al. (1999) (16–17.5°C). It was just reverse in April–May, when the RH decreased to 64% with an increase in minimum and maximum temperatures (21–42°C) and mangiferin content (9.38%); thus, the fungal population (7 c.f.u. g^{-1} plant material) as well as development of malformed shoots was reduced to a minimum level. In July–September, the range of temperature (25–32°C) and mangiferin content (4%) receded but humidity (87%) increased leading to the increase in the number of propagules of *Fusarium* (126 c.f.u. g^{-1} plant material) and malformed shoots. The range of population of eriophyid mites per malformed bud was recorded maximum (0–104) in March, very low (0–39) in June and intermediary (0–75) in July (Prasad et al. 1965).

Chand and Chakrabarti (2003) recorded in details the interactions between meteorological conditions with the pathogen and the host and the sequences of resultant manifestations of the disease symptoms (Fig. 6.1). Floral buds were inoculated at an early stage of inception i.e. last week of November. The buds remained as such until the end of next January. From 23.1.99 to 7.2.99, mangiferin content declined with subsequent logarithmic increase in the fungal population. During the last week of January to first week of February, temperature became mild (8–19°C) and RH was high (87%) and malformation symptoms on floral buds became apparent for the first time. Within next fortnight, the manifestation of disease symptoms became

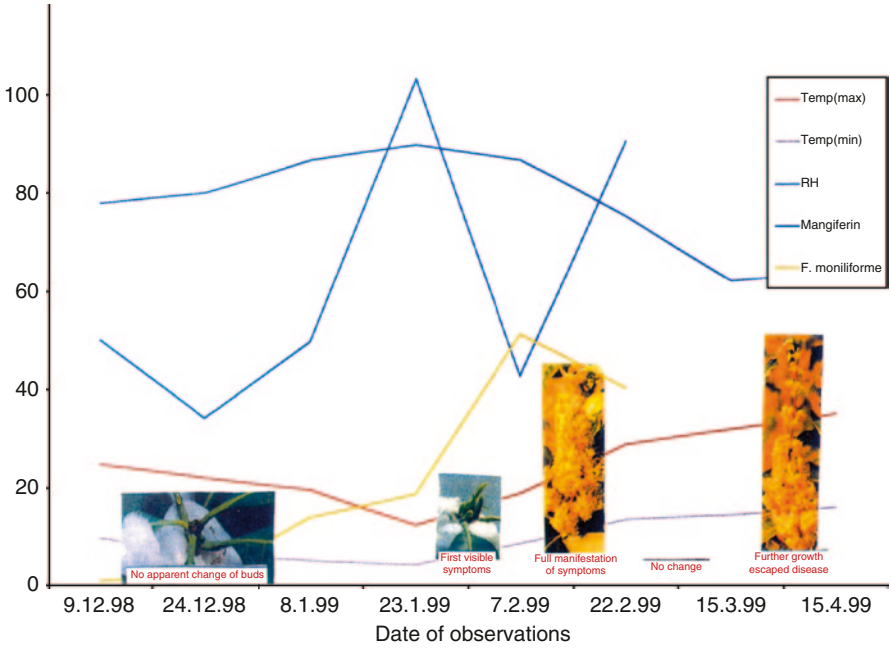

Fig. 6.1 Effect of temperature, RH, mangiferin content and *F. moniliforme* var. *subglutinans* population on development of floral malmation in mango cv. Amrapali

complete. The process was reversed when temperature started increasing in the end of February. Since at the end of February further developed parts of the artificially induced malformed panicles escaped the disease and looked normal (Fig. 6.1). In May–June, the malformed panicles dried up. Vegetative shoots that developed from the base of these dried malformed panicles appeared normal. In the first week of August when climate was hot and humid, cottony growth of pinkish mycelia of *F. moniliforme* became conspicuous over remnants of the yester year's malformed panicles and the buds developed during this period appeared malformed. In November, temperature declined and RH increased. Mild temperature (8–27°C) and high RH (80.14%) favoured proliferation of the inoculum on the host surface while low mangiferin content could offer little resistance to the penetrating hyphae. By November, the infected buds of August developed into prominent malformed shoots.

Development of malformed shootlets in nature since its first appearance in mid June to till October when rate of emergence of new shootlets stopped was recorded (Chand and Chakrabarti 2004). Simultaneously Pandey et al. (2005) recorded the rate of formation of conidia over the necrotic malformed panicles. In nature, number of conidia on necrotic malformed panicles started from April. There was a gradual increment and then it reached a peak in the month of July when range of temperature was 25–30°C and RH 85–92%. Thus, from mid June to end of July there was a continuous increase in number of malformed shootlets. Maximum increment in number of malformed shootlets was recorded during 16–31 July. Number of days with optimum temperature (8–27°C) and RH (85%) and total rainfall showed significant positive correlation with increment of the diseased shootlets. Since August, a decline in multiplication of conidia was recorded. At the same time, the flushing process was over. Thus, there was no further increase in number of malformed shootlets in spite of prevalence of favourable weather. This was presumably due to the lack of infection sites i.e. tender emerging buds.

From the infected mango buds during July–August the percentage of malformed shoots developing up to first week of October, in between October and early February, and February to early May were 25, 42 and 32% respectively. This suggests that the buds infected during July–August remained infectious throughout and developed into malformed shoots during the flushing period. The prevailing weather conditions during that particular flushing period determined the number of malformed shoots (Pandey 2003; Chand and Chakrabarti 2004).

Non-target Effects of Agro-Chemicals on Malformation

Besides malformation, mango plants also suffer from various diseases, insect pests, and physiological disorders. Thus, a wide range of alien chemicals, many times used at an overdose, is administered the mango plants every year. Indiscriminate use of agro-chemicals is known to result into breaking out of new diseases called the iatrogenic diseases. However, sometimes non-target effects of the agro-chemicals

may be advantageous. Kumar (2007) and Kumar and Chakrabarti (2007, 2009) reported effects of six agro-chemicals viz. monocrotophos, dimethoate, sulphur, streptomycin, borax and naphthyl acetic acid (NAA) commonly and widely used on mango plants on the population of *Aspergillus niger* (an antagonist to *F. moniliforme* var. *subglutinans*) commonly present in mango phyllosphere and defense related metabolite (mangiferin) and enzymes (peroxidase, polyphenol oxidase) of *M. indica* in the context malformation disease. Of the six agro-chemicals tested, monocrotophos and dimethoate after repeated applications drastically reduced the population of *A. niger,* seriously affected mangiferin producing capacity of the host and inactivated its peroxidase and polyphenol oxidase enzymes. Plants took long time to go back to normalcy even after spraying was discontinued. The crop remained safe till the insecticides were applied. But once discontinued, it caused severe malformation. Sulphur did not show any deleterious effects on the antagonist or plant defense system but it increased the population of the pathogen. This may cause problems in the long run by tilting the balance of phyllosphere mycoflora of plants. Streptomycin increased the pathogen population and reduced that of antagonists. Borax application increased *A. niger* population but reduced mangiferin content and lowered the activity of peroxidase and polyphenol oxidase. NAA adversely affected *A. niger* population. The above observations corroborated an earlier report by Chadha et al. (1979). In a field trial they recorded the incidence of malformation on plants treated with dimethoate and monocrotophos to be 0.89 and 2.79% respectively in the first year of the treatment while in the control it was 13.16%. But in the second year, the disease incidence on dimethoate and monocrotophos treated plants was increased to 7.52 and 4.34% respectively while on control plants the same was reduced to 9.97% only. Recently, carbendazim has also been reported to decrease phenol content and the antagonist population of mango (Gaur and Chakrabarti 2009). The results further suggest that prolonged use of insecticides may be counterproductive. Thus, during formulation of a control strategy and package of practices, instead of focusing on one problem only, overall health and productivity of the crop has to be taken into consideration.

Disease Cycle

The malformation disease symptoms of mango were variously described as an abnormal inflorescence (usually referred to heavy type malformed panicle) (Singh and Chakravarti 1935), bunchy top (Nirvan 1953), the die-back (the late stage of vegetative malformation) (Vaheduddin 1953) and blossom blight (late stage of light type malformed panicle) (Chakrabarti and Ghosal 1989), although the causative agent for the said disease, malformation, was identified as *F. moniliforme* var. *subglutinans.* These apparently discrete disease syndromes are, in fact, inter-linked and can be expressed through a disease cycle (Chakrabarti and Ghosal 1989). The conidia, the infecting unit, of the pathogen, are produced profusely over dead necrotic

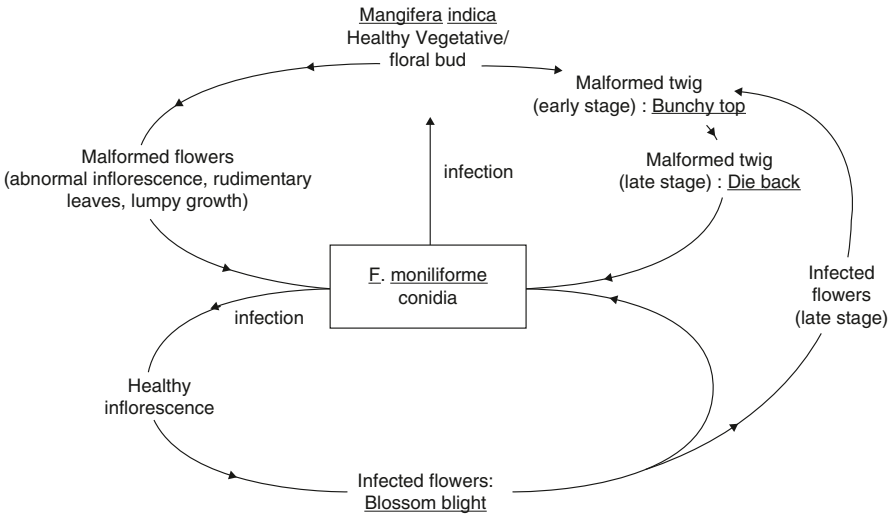

Fig. 6.2 Disease cycle of mango malformation. (Chakrabarti and Ghosal 1989)

malformed shoots and panicles which are carried to the infection site, developing buds, by the mite. Emerging buds are tender, contains low amount of the defense chemical mangiferin but carbohydrate in high quantities that serves as food for the pathogen. Thus there could be no better site than these buds for invasion. The host produces abnormal metabolites and the pathogen various toxins. The resultant of such interaction is that the buds are ultimately transformed either into malformed shoots or panicles. Later mangiferin increases to a cytotoxic level. Due to the effect of high content of mangiferin and phytotoxins the malformed tissues undergo necrosis. At this stage mangiferin is oxidized into polymeric quinone which does not have any perceptible anti-*Fusarium* activity. The surviving propagules grow over the dead necrotic cells and produce new crops of conidia to initiate fresh infection. To postulate the disease cycle, information on temporal disease progress at each of the above mentioned stages is a pre-requisite. Therefore, the disease syndromes were reproduced over the cultivar Banarasi Langra by repeated spraying with fungal suspension under highly humid conditions as mentioned in the section "Artificial inoculation" and different stages of the disease manifestations were recorded in details and finally integrated into an infection chain (Fig. 6.2).

Kumar and Beniwal (1992) linked the different developmental stages of the malformation symptoms together and proposed the following disease cycle (Fig. 6.3).

Noriega-Cantu et al. (1999) integrated the sequence of development of malformation symptoms on different plant parts in naturally infected plants in Mexico as follows. (a) In mid-June, the vegetative shoots emerge and during this period, the pathogen heavily infects the apical meristems of the tender vegetative buds. (b) In October, first visible symptoms as malformed vegetative shoots appear; these vegetative shoots produce malformed panicles in the forthcoming flowering season. (c)

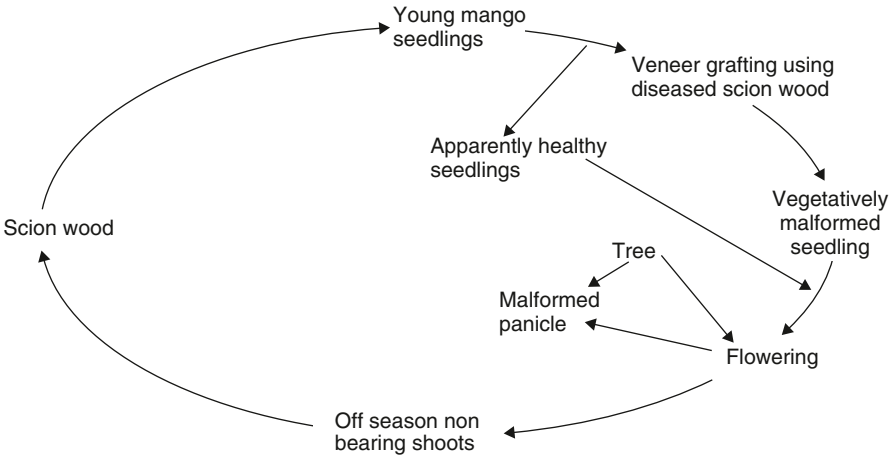

Fig. 6.3 Disease cycle of mango malformation. (Kumar and Beniwal 1992)

In December–January, full bloom occurs. Some healthy panicles may be infected at this stage and remain unproductive (referred to as blossom blight by Chakrabarti and Ghosal 1989). (d) In January–February, second vegetative flush takes place. (e) In mid February to mid March, there is another spate of infection by the *Fusarium* over the newly developed spring flush. (f) In April–May, deformed vegetative and floral shoots remain in the trees and serve as sites of multiplication of the fungus and source of infection entity for the fresh infection sites. Based on the above information, we envisage the following disease cycle (Fig. 6.4).

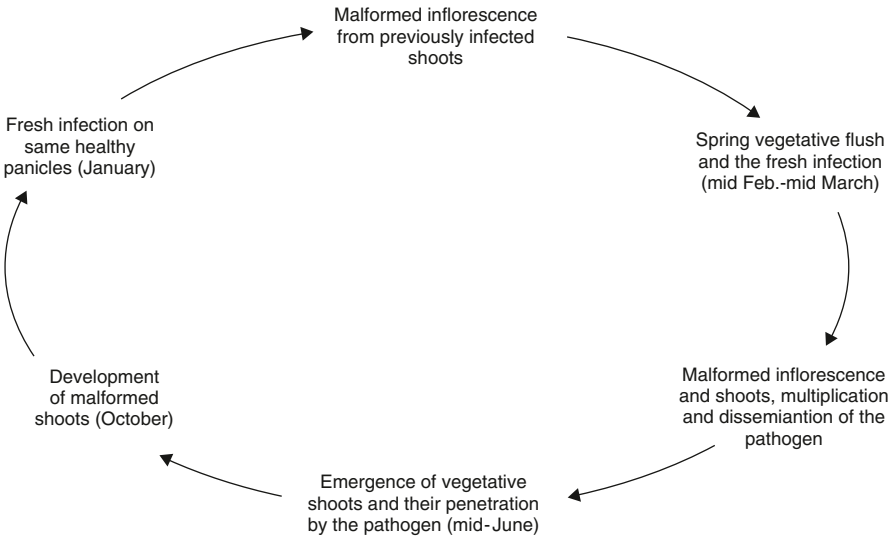

Fig. 6.4 Disease cycle of mango malformation drawn according to Noriega-Cantu et al. (1999)

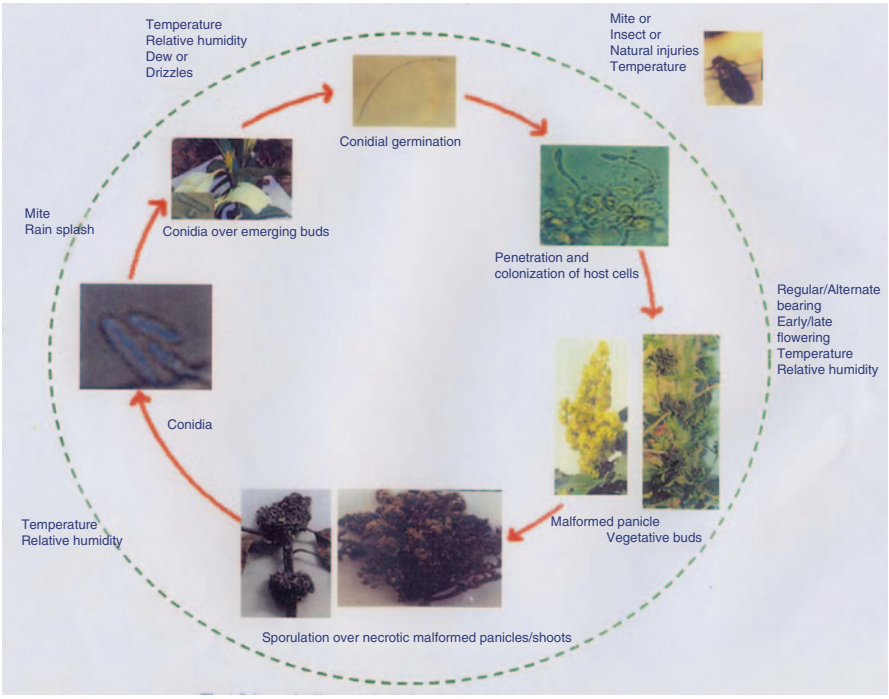

Fig. 6.5 Disease cycle of mango malformation. (Pandey 2003)

Recently, Pandey (2003) modified the disease cycle of Chakrabarti and Ghosal (1989) after Shrun (1978). The modified disease cycle depicts the dynamics of the malformation disease, interlocking effects of different parts of the infection chains and effects of external factors such as environmental parameters, vectors etc. on the temporal progress of the disease (Fig. 6.5). Shrun (1978) envisaged that the epidemic system is comprised of several states (states of variables) that represent the stages through which the pathogen passes as the disease progresses, for example, the propagules, the invasion, and development of malformed shoots and panicles. The rate of transformation from one state to another is influenced by the external variables. These comprise the factors of the host environment. The external variables are not part of the system but they act over the state variables. They may either slow down or accelerate the progression from one state to another.

Patterns of the Epidemic

Patterns of the disease progress curves of regular (Neelum), semi-regular (Mallika) and alternate bearing (Banarasi Langra and Himsagar) cultivars are presented in the Fig. 6.6. In alternate and semi-regular cultivars, the disease progress

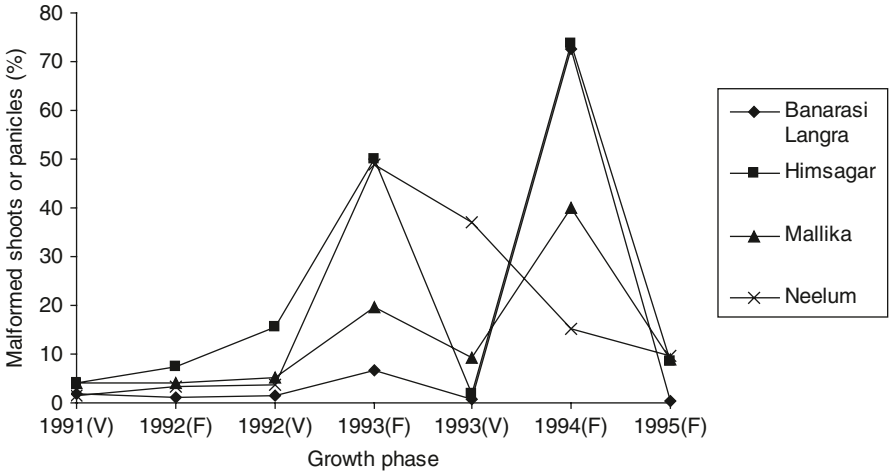

Fig. 6.6 Patterns of mango malformation epidemic

curves may be broadly divided into four phases. At the first phase (initial to the 'take off' phase) the disease progresses gradually. At second phase a small peak (first peak) is formed. In Neelum the peak at this stage reaches its maximum. Then at the third phase, the curves decline gradually in Neelum but sharply in others. Later in alternate and semi-regular cultivars, the curves shoot up to their highest to form a second peak. But in Neelum no second peak is formed; the disease progress curves decline further. Thus, the disease progress curve of Banarasi Langra is typically bimodal with small initial peak. Similar bimodal curve is noticed in Mallika. In Himsagar, the curve is initially sigmoid (S-shaped) but later it changes towards bimodal. However, typical sigmoid curve is noticed in Neelum. The sigmoid disease progress curve and variable infection rates suggest that the disease is polycyclic and the pathogen is polyetic. The bimodal polycyclic disease curve of the alternate bearing cultivars is the characteristic for the disease affecting different (shoots and panicles) of the plant at different times. It reflects discontinuities in the infection process. The sigmoid curve in regular bearing Neelum indicates undisturbed progress of the disease (Kranz 1978). In regular bearing cultivars new shoots and panicles (site of infection) are available every year whereas in alternate bearers panicles are available mainly in 'on years'. It is well known that the shape of sigmoid disease progress curve and bell shaped rate curve (e.g. Neelum) may be affected into asymmetrical one by intermittent availability of the inocula or irregular sequences of growth flushes (as in alternate bearing cultivars) (Kranz 1978). Such type of sensitivity of the disease dynamics to the host factors is usually governed by horizontal resistance (Day 1978). In all the cultivars the slowing down of the disease progress curves is recorded after the disease is mounted. Van der Plank (1975) made similar observations in case of *Fusarium* infection in cotton. This might be due to antagonistic interactions

between spores for the infection court (Chakrabarti et al. 2005). Recently it has been observed that with the increase in incidence of malformation, the pH of the cell sap of the infected plants increases which concurrently reduces the fungal population (Chakrabarti and Kumar 1999). It may be mentioned here that *F. monilifomre* var. *subglutinans* prefers a low pH for optimal growth (Kumar 1992). Thus, the epidemic stage (logarithmic growth phase) might be initiated with small amounts of initial inoculum and once the tissues are infected they remain infectious throughout. The epidemic, after 4–5 years of epidemic phase, enters into endemic stage (Chakrabarti et al. 2005).

Endemic Stage

Endemicity is the state of balance in host-patho systems. After continuous increase of the disease incidence for 4–5 years (epidemic stage), a state of host-pathogen equilibrium is attained i.e. endemic stage. In epidemic phase, production of daughter infection per parent infection (iR) is more than one but at endemic stage iR declines below one. But the increase in spore density inhibits the conidial germination. Besides, with increase in disease severity, the host also enhances production of its defense compounds (mangiferin) which in turn affects the colonization by the pathogen resulting in a reduction in the disease incidence. Therefore, in mango malformation, the more is the mother malformed panicles, the lesser is the daughter infection (progeny). With increase in the disease percentage, the epidemic approached faster towards being leveled off (endemicity). In 'off year' production of smaller number of panicles reduces the infection site; consequently the number of progeny (daughter infection) vis-à-vis the inoculum potential for the next crop season. In alternate bearing cultivars of Dashehari and Langra, endemicity was attained earlier and asymptote *L,* at which the disease would level off, was higher indicating that fitness of these cultivars was less affected even in constant presence of the disease. At endemic phase, the catastrophic initial epidemic of mango malformation is abated without intervention of fungicides. The pathogen, *F. moniliforme* var. *subglutinans* is with polycyclic reproductive capacity, capable of thriving under varied conditions and stress related adaptation lead to more or less permanent changes in it. Thus, the crop-patho system, due to the highly adaptive and mutable genes of the pathogen, is unstable and prone to the epidemic. Due to low levels of disease incidence vis-à-vis the inoculum potential at the endemic stage, defense system of the host plant could prevent the break out of the disease in epidemic form. Thus, a state of host-patho equilibrium was attained. In alternate bearing cultivars the sequential (seasonal) discontinuity of host tissues (panicles) in the 'off year' reduced the inoculum potential drastically; hence thus provided an added advantage to reactivate vertical resistance of the host more frequently and within a short spell of the epidemic phase (Fig. 6.7).

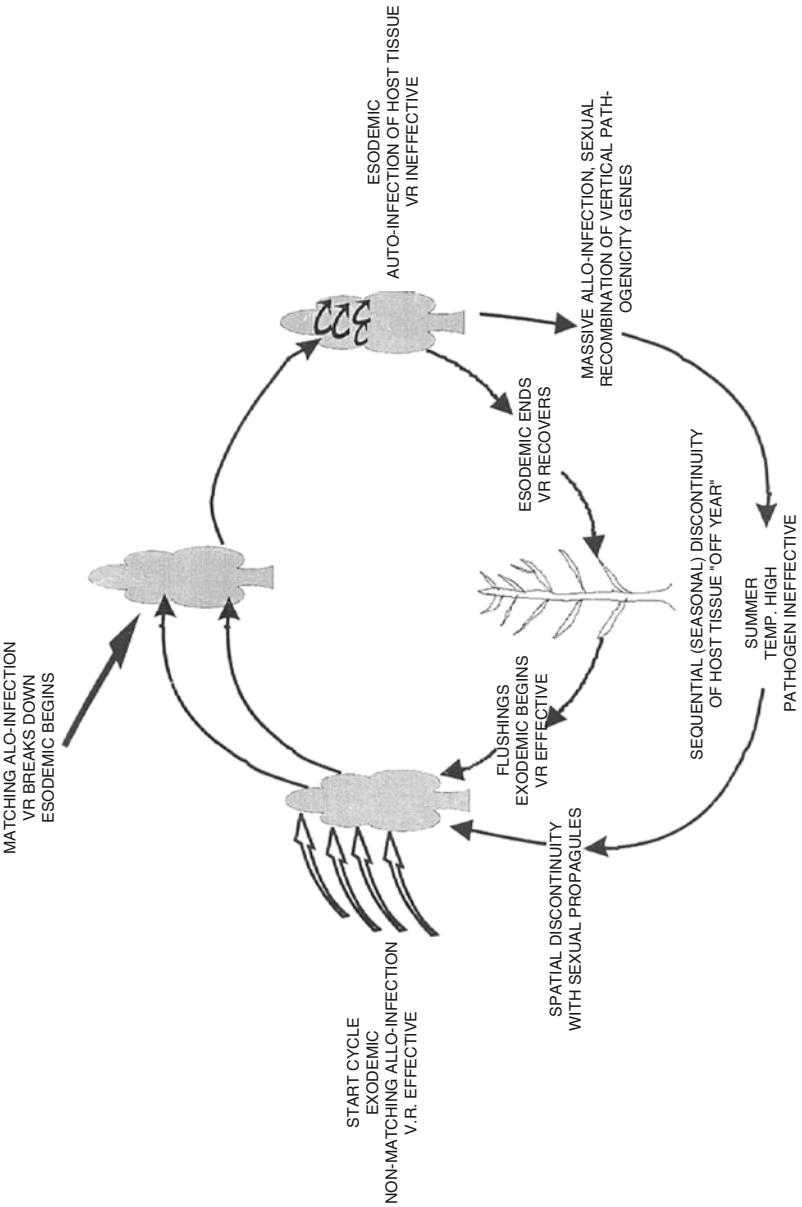

Fig. 6.7 Probable mechanism of attaining of endemic phase in mango against malformation

Dispersal of the Disease

The disease elicits both temporal and spatial spread both between plants and within plants.

Dissemination from Plant to Plant

During one 3-year study on the incidence of floral malformation in young Dashehari trees (8–10-years-old), only one new infection was recorded in a grove where 70% plants were malformed (Kumar and Beniwal 1992). In a separate study the spatial patterns of spread of the disease among genetically diverse cultivars of mango were investigated (Kumar and Chakrabati 1997b). Experiments (Fig. 6.8) were conducted in four separate blocks, one block of each of Dashehari, Himsagar and Mallika. The fourth one consisted of an unequal mix of Gilas, Mallika and Banarasi Langra and Dashehari. The disease gradients in all the blocks at an early stage of infection were hyperbolic. Disease incidence decreased steeply within a short dis-

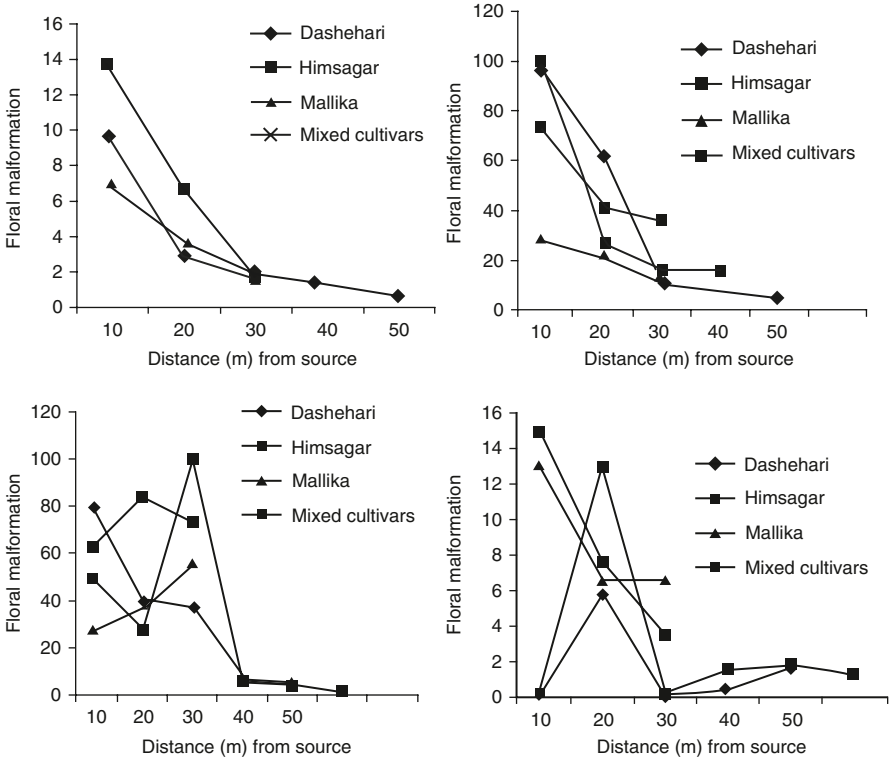

Fig. 6.8 Disperasal of malformation from plant to plant

tance until they reached zero (e.g. in mixed cultivars block). In the next year, the curves in Dashehari and Mallika tended to be more flat near the source. In the next year, the disease gradient curve in Mallika elicited reverse trends to that of the first year of the experimentations. Almost similar reversal was noticed in Himsagar. In Dashehari, the curve near the source became more flattened. In mixed cultivars block, the reverse trend of the gradient curve was apparent among Gilas to Banarasi Langra. Infection spread to two more Dashehari plants. These results show that the disease spreads in a stepwise progression i.e. plants receives the inocula from its immediate neighbour. Hence, the disease spreads over short distance only. Kumar et al. (1993) also observed that the disease spreads slowly from infected to healthy seedlings/trees. A healthy tree adjacent to a diseased tree may remain healthy for many years. The possibility of air transmission of the disease was investigated by several workers. Varma et al. (1971) used rotary trap in an infected orchard for six months (November to April) to check the air movement of the spores but no *Fusarium* spore was trapped. However, they did not rule out aerial transmission of the fungal spores. Later, Noriega-Cantu et al. (1999) succeeded to trap air borne conidia of *Fusarium* sp. with spore trap operated daily a week per month during the vegetative stage and daily during flowering. But they were not sure whether the spores trapped in the mango canopy were exclusively attributed to this species. The pathogen also seems to be splash dispersed. However, the fungus sporulates on the surface of dying malformed branches (Varma et al. 1971; Chakrabarti and Ghosal 1989) and the conidia serve as the source of secondary infection. One of the causes of wide and erratic distribution of the disease is inadvertent propagation and distribution of malformed plants as has been discussed in earlier Chap. 2 (p. 11). Van der Plank (1975) also observed highest rate of development of epidemics on vegetatively propagated crops. The slow dissemination of the disease from plant to plant indicates that the causal factor is localized and spreads slowly. Kumar et al. (1993) found no geometric increase of disease spread from tree to tree with the increase in inoculum potential. However, recently, Gamliet-Atinsky et al. (2009b) reported that the spatial patterns of primary infections in a heavily infected orchard corresponded with a typical dispersal pattern caused by air borne conidia propagules. Significantly higher number of conidia were detected in May and June than in April. A peak in trapped airborne conidia was detected in May and June. Higher number of conidia were trapped when RH values were low (<55%). They claimed that air borne conidia served as the primary means of inoculum spread. However, after establishment of the secondary source of inoculum within these perennial plants, the hyperbolic disease gradient curve becomes flattened near the source. The gradient became flatter as the rate of infection becomes faster (Van der Plank 1960).

Kumar and Beniwal (1992) monitored the incidence of vegetative malformation in the same mango nursery for two consecutive years. The affected trees were found to be localized in a particular zone of the nursery. The infection spreads slowly but there was no directional trend in the spread of the infection. Similarly, spread of floral malformation in a 15-year-old orchard of 81 plants (at 10×10 m distance) belonging to different varieties was recorded for continuous 4 years (1991–1994) (Kumar and Chakrabarti 1997b) (Fig. 6.9). It was found that in 1991, only one

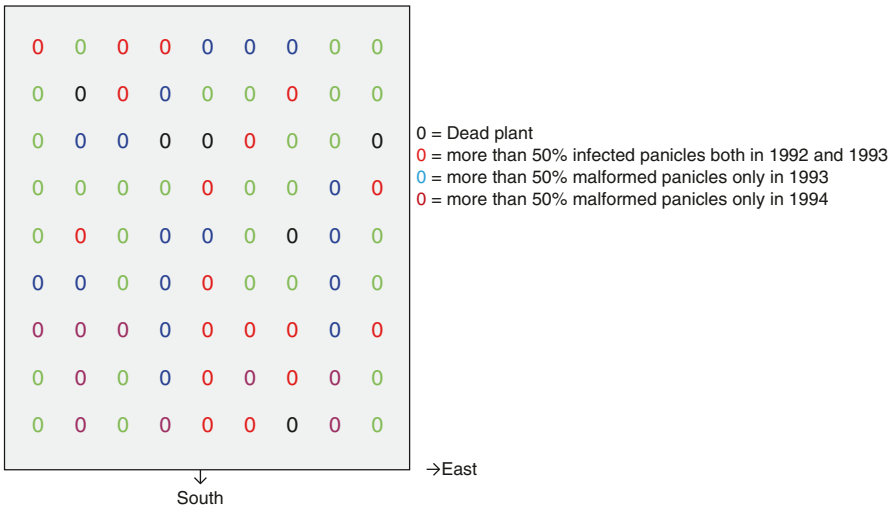

Fig. 6.9 Incidence of floral malformation in the same orchard during 1992–1994

high infection zone of three plants with more than 25% malformed panicles was developed in the form of a patch at the southern boundary side of the orchard. Next year, two similar patches appeared near the initial disease zone. Subsequently, in the third and fourth year, scattered appearance of five and two new high infection zones were recorded respectively. The results clearly indicated that the disease appears in patches. In each patch, there were three to five highly infected plants. From the sequence of appearance of the infection patches, it appears that the disease moved from south towards the north side and from boundary area towards inside of the orchards. To confirm the south to north direction spread of the infection, in a replicated trial, the percentage of malformed inflorescence developing on all the four directions (east, west, north and south) in a 12 years-old orchard growing susceptible mango cv. Sunderja, were counted (Chakrabarti, unpublished). The plants were at the extreme border of the east side of the orchards and had access to sunlight most of the early part of the day. The average percentage of malformed inflorescence was maximum (54.79%) on the north side of the plants followed in descending order by west (43.49%) and southern (39.27%) branches of the trees. The minimum was recorded on the east side (29.94%). It may be pointed out that Chadha et al. (1979) earlier observed that the malformation was more on periphery than inside of the trees.

Spread Inside the Plant

Singh et al. (1961) recorded the incidence of floral malformation for consecutive 5 years (1954–1958) on very young plants (5–9-years-old). A gradual increase in

the incidence from 22 to 30% was recorded. Kumar et al. (1993) observed that in a tree only few branches continue to bear malformed inflorescences year after year. But percentage of malformed panicles on individual trees varied in subsequent years (Kumar and Beniwal 1992). Chakrabarti et al. (2005) also noticed great variation in the incidence of floral malformation on an individual tree irrespective of alternate or regular bearing cultivars in different years. For example, in an alternate bearing cultivar, Himsagar, the number of malformed panicles at the time of initiation of the experiment was 42, in the next year it increased to 252 followed by a reduction to 92 in the third year. Similarly, in regular bearing cultivar, Neelum, the number of malformed panicles at the starting was 44 that increased to 310 in the next year but in the third year it was reduced to 91 only. These results definitely suggest that the disease may spread inside a tree very fast. Moreover, this spread is possibly effected by the mites as has been discussed in the earlier Chap. 5.

Adaptability of the Pathogen

There was a popular perception that the disease could not occur in the States situated at the coastal regions of India. This might be due to constant high temperature in the region and unlike northern part of the country, fluctuation in temperature between winter and summer months was not extreme.

In 1995, a survey was conducted in the mango belt of West Bengal, a coastal state of India (Chakrabarti and Kumar 1997). The orchards were having both young (7 years-old) and grown up trees (20 years-old) of the traditionally grown cultivars of West Bengal viz. Himsagar, Bombay Green, Rani Pasand and Langra. In these orchards, plants of the hybrid cultivars of Amrapali and Mallika were introduced five years before. Both the hybrids are highly susceptible to the malformation disease and they were procured from northern India, the hot bed of the malformation disease (Gaur and Chakrabarti 2009). The survey revealed that in 5 years-old plants of Amrapali grown in mango belt of West Bengal, about 22% plants did not produce any flower (although it is a regular bearing hybrid), about 8% plants produced bare healthy panicles and in the rest of the plants floral malformation were recorded at varying degrees. Similarly, in the cultivar Mallika, approximately 73% plants were without any flower while in the rest, the inflorescences were malformed. Plants of the cultivars Langra and Himsagar, adjacent to the Amrapali were found to bear 0.1–5% malformed panicles. Since 100% plants of cvs. Amrapali and Mallika in West Bengal orchards were malformed, it is presumed that the plants were infected at nursery stage i.e. five years earlier. The pathogen introduced with the planting materials in new agro-climatic conditions was capable of causing infection and producing disease symptoms. From the source plants, the disease was found to spread to the local cultivars already growing in orchards and were so far free from the disease. However, in the agro-climatic conditions of West Bengal the manifestation of the disease symptoms was less severe i.e. malformed panicles were mostly of ‘loose type’ and they sustained only up to March. In comparison to this, malformed

panicles in Uttar Pradesh were of 'compact type' and survived up to June–July. The physiologically specialized strain of *F. moniliforme* being introduced to the West Bengal underwent some significant morphological and biochemical changes indicating loss of virulence to some extent. Varma et al. (1974) also noticed that the malformation symptoms of mango in coastal areas of Kerala and Kanya Kumari of southern India considerably differ from those of the northern India.

Disease Forecasting

The epidemic system is comprised of several stages through which the pathogen passes as the disease progresses, for example, the propagule formation, dissemination, the host invasion leading to disease manifestations. Shrun (1978) described these stages as state of variables. The rates of transformation from one state to another are influenced by external factor variables. These comprise of factors of host like host age, its bearing habit, and time of flowering; factors like climatic parameters and the propagule dissemination agencies like mites. In developing a forecasting system, every phase of the host-pathosystem is to be modeled separately starting from infection proceeds through invasion, incubation, symptom development to sporulation and dispersal. Thus, to quantify or model mathematically the changes in disease level with passage of time, all components of the disease cycle are used. This is known as analytical approach to modeling. In developing a forecasting system relying only on infection or weather variables, although common, may not lead to sufficiently accurate predictions.

Mathematical Modeling

Rajan and Majumder (1995) analyzed growth of mango panicles in terms of length of its main axis by using asymptotic curves. The monomolecular, logistic and Gompertz models were tested for fitting mango panicle growth data. The best fitting model was selected on the basis of low limiting values of the panicle main axis (A) and error sum of square (ESS) and high value of multiple coefficient of determination (R^2). Malformed panicles exhibited a growth pattern similar to normal ones with significantly less absolute growth rate and 'A' values. The monomolecular function was found unsuitable for describing growth progression from initial growth phase. Rather it might be useful in describing growth in later stages of the panicle growth. The logistic and Gompertz functions were very close to the primary data and described the variation in progression of growth of malformed and normal panicles with high accuracy. Pandey (2003) also observed that growth patterns of malformed and normal panicles in terms of length and weight were similar. He used Sigmoid, Weibull and Gompertz functions for growth analysis. Considering the above mentioned criteria for the best fitting models, he found the Sigmoid and

Gompertz models are appropriate for describing progression of length as well as weight of both malformed and normal panicles. Noriega-Cantu et al. (1999) used monomolecular, logistic, Gompertz and Weibull models for temporal characterization of the malformation epidemics of the plants treated with various plant protection measures. Gompertz and monomolecular models were found to be most suitable for describing the epidemics when the disease incidence was high and low respectively. Weibull model adequately described all the epidemics in both growing cycles. Pandey (2003) fitted the observed temporal progression data on conidia production of *F. moniliforme* var. *subglutinans* on dead necrotic malformed panicles with three different forms of curves i.e. linear, quadratic and cubic. Cubic model was found to give the best fit. From the cubic curve optimum incubation period (81.9 h) for maximum conidia production and for shortest class interval (SCI) for top 100% conidia production (68.7–94 h) was also determined. Similarly, the observed data on effects of temperature on germination of conidia were fitted into three different curves i.e. linear, quadratic and cubic. The R^2 value was the maximum (0.986) in the cubic model. The optimum temperature for conidia germination (32.3°C) and shortest class interval (SCI) for top 100% conidia germination (27.5–36.3°C) was also determined from the best fitted curve.

Prediction Equations

Prediction equations were worked out by various workers based on weather, host and pathogen variables and their combinations.

Weather Variables

Multiple regression analysis (MRA) was performed to find out the functional relationship of number of conidia production per gram necrotic panicle in nature during April to August with average temperature and relative humidity (Pandey 2003; Pandey et al. 2005). The prediction equation developed was: $Y = 293.6 - 101.9X_1 + 178.4X_2$ where X_1 and X_2 were average temperature and RH respectively. Multiple coefficient of determination (R^2) shows that combined effects of weather variable favour the conidia production up to 91.97%. The model was validated using the goodness of fit test. The χ^2 value (8.46) confirmed the validity of the model. MRA of the functional relationship of number of malformed shoot produced with optimum range of temperature (25–30°C) and moisture (>85%), number of rainy days and total rain fall (in millimeter) yielded the following prediction equation: $Y = 10.234 + 4.336X_1 - 0.243X_2 - 0.141X_3$, where X_1 = number of days with optimum range of temperature and moisture, X_2 = number of rainy days and X_3 = total rain fall (Pandey 2003). A unit change in optimum range of temperature and moisture influenced the disease incidence up to an extent of 4.336 units in positive direction and number of rainy days by 0.243 units followed by total rainfall

by 0.141 units in the opposite direction. Multiple correlation coefficient determination (R^2) between number of malformed shootlets and weather variables indicated that 83.66% changes in disease incidence were caused by the weather parameters. Partial correlation coefficients of total rainfall (−0.720) and number of days with optimum range of temperature and moisture (0.826) did not differ much from the multiple correlation coefficient determination; thus, these two variables seem to have effective relationship with the disease incidence (number of malformed shootlets) and may be used as predictors.

Host Variables

The two host variables commonly used for modeling were host age and internal tissue constituents.

Host Age

The pattern of changes in incidence of vegetative and floral malformation with time, from a set of experimental data obtained on the cv. Dashehari at the university orchard, Faizabad, eastern Uttar Pradesh, India was expressed mathematically (Pandey et al. 2003). The observed data showed that the disease initially increased very rapidly followed by a curvilinear decrease. These data were fitted to a set of polynomial equations. The values of R^2, Residual SS, and Durbin Watson statistic were examined to test goodness of fit of the model. The R^2 value (0.981) in quadratic model was the highest. A regression equation, $Y = 111.892 - 9.989X + 0.252X^2$, where X and Y were the host age and the number of malformed shoots respectively, was derived. The simulated curve from the quadratic equation was then compared with the observed disease progress curve. The observed values for vegetative malformation progression fluctuated around the predicted values with non-significant error. χ^2 value (1.355) also confirmed the validity of the prediction model.

The observed data on the progression of floral malformation of the cv. Dashehari with the host age also followed similar pattern to that of the vegetative malformation and the quadratic model here also gave the best fit. Based on the results of the regression analysis the prediction equation developed was as follows. $Y = 17.605 + 7.775X - 0.329X^2$, where X and Y were host age and number of malformed panicles respectively and R^2 value was 0.989. The χ^2 value (0.112) also supports that the model is adequate to describe the progress of the floral malformation in different age groups of plants. This model was tested using a set of data on incidence of floral malformation on the cv. Dashehari of different age groups of plants recorded in Lucknow, near Faizabad in 1975 (Chadha et al. 1979). The calculated values from the above mentioned quadratic equation and the observed values in orchards of Lucknow in 1975, showed close resemblance and thus validated the proposed prediction model.

Using a polynomial model optimum host age for the maximum incidence of floral malformation was determined. Thus, the optimum host age was found to be 11.3 years when the floral malformation reached its peak. The annual rate of progress during this period was 15.2% while rate of decline was 11.4%. Similarly, vegetative malformation was maximum when the trees were ca. 5 years. Thereafter with increasing age, the disease decreased at an average rate of 10.82% per annum.

Biochemical Constituents

Maksoud and Haggag (1994, 1995) evaluated several models using linear multiple regression to represent the relationship between leaf mineral nutrients (Ca, Mg, Fe, Mn, Zn, and Cu) or biochemical constituents like phenol, indoles and gibberellins of panicles and the percentage of malformed panicles in mango cvs. Pairi and Taimour.

The Pathogen Variables

The pathogen variables tested included primarily the inoculums potential.

Inoculum Potential

An experiment was conducted in 10 year-old 23 plants of 'Chausa' mango cultivar during 1996–1997 and 1998–1999 at Rudauli-Sohawal mango belt, Faizabad, Uttar Pradesh (Chakrabarti et al. 2003) using both internal constituent host variables and population density of the pathogen. In November (time of flower bud initiation) the amount of phenol (mangiferin), C:N ratio, nutrients (zinc, copper, iron and manganese) of leaves attached to the apical buds, amounts of auxin of the emerging buds and population density of *F. moniliforme* var. *subglutinans* of buds before flowering were estimated. Besides number of malformed shoots developed during June–October, and average number of total and malformed inflorescence produced in the following crop season (March–April) were also counted in October and May respectively. The correlation matrix for the variables showed that the effects of the biochemical constituents of the host cells on the disease development varied with the fluctuation of their concentrations in different crop seasons. Only the relationship between vegetative and floral malformation, positive and significant, was consistent throughout. The relationship between the two variables was expressed by the equation, $Y = 2.136 + 0.697X$, where X and Y were number of malformed panicles and vegetative shoots respectively. The R^2 value $= 0.946$ expressed appropriateness of the fitted relationship. With the help of this equation, the probable numbers of malformed panicles in the subsequent years were predicted. The observed and predicted values of the floral malformation showed considerable similarities. The values of χ^2 also confirmed the validity of the forecasting equation. Thus, simple

counting of number of malformed shoots present prior to the onset of flowering process in October may help by using this predicting equation to forecast the number of malformed inflorescence to be produced in the flowering season in 'Chausa' under the agro-climatic conditions of the particular zone in Uttar Pradesh.

Expert System

Attempts have been made infrequently to device an expert system for optimizing plantation yield in general and malformation management in particular.

To predict the disease incidence in any State of India and to suggest appropriate IPM strategy, a computerized decision support system, Expert System for Management of Malformation Disease of Mango (ESMMDM) has been developed. The expert system is based on long term researches on the etiology, epidemiology and management trials both under laboratory and field conditions (Chakrabarti and Chakraborty 2006, 2007; Chakraborty and Chakrabarti 2008a, b). The process for the expert system is broadly divided into 4 stages. It begins by confirming the occurrence of the disease in the orchards. The second stage is the user interface in which a series of questions were designed in simple native language. The questionnaire is supported by combo boxes and radio buttons. The questionnaires have multiple choices added by coloured photographs of the symptoms. User is required to select one of the options from a list of drop down menu box. Depending upon the input answers, the next question will appear on the screen. At the third stage the engine generates three treatment packages prescribed as high, medium and low intensity control. For this inference mechanism a fully deterministic algorithm was developed. The information obtained as the user response act as the input to the algorithm. This inference making algorithm was implemented in Visual Basic 6 using simple constructs like IF, Then… Else ladders. This algorithm is responsible for all the reasoning and decision making activities in the software. Finally it generates a report of the current case which includes the details of the symptoms, epidemiology and treatment packages. The report is stored in a file and can be opened in any text editor like Notepad or WordPad and is in printable form. Preliminary testing of this system was done involving small clientele groups in and around Uttar Pradesh. This software was found to enhance the performance of farmers and extension personnel, reduce time required to solve the problem without waiting for an expert advice and makes mango cultivation more efficient and profitable.

Chapter 7
Varietal Susceptibility

The varietal response of numerous mono- and ploymbryonic mango cultivars towards malformation have been evaluated by various workers. But all these evaluation trials were conducted in field under epiphytotic conditions. The susceptible or resistant reaction of a cultivar is greatly influenced by the age of plants, flowering behaviour ('on' and 'off' year phenomenon, early or late flowering habit) and agro-climatic conditions. Besides, for measuring the disease intensity, no single method has been followed. Thus, the disease incidence on different varieties reported by different workers under various agro-climatic conditions varies considerably (Table 7.1) and hardly comparable.

Singh et al. (1961) under Saharanpur, UP conditions recorded no disease on cultivar Taimour but El-Ghandour et al. (1979) in Egypt found this variety to be susceptible. Similarly, Prasad et al. (1965) and Varma et al. (1971) reported Chausa, Dashehari and Langra as highly susceptible. The report was confirmed by Sharma and Badiyala (1990) from Himachal Pradesh who recorded highest incidence of malformation in Dashehari followed by Malda, Chausa and Langra. Contrarily, Dang and Daulta (1982) and Singh and Jawanda (1961) observed Dashehari as less susceptible. Schlosser (1971) noticed some disease tolerance in Langra. Jagirdar and Shaik (1968) also categorized the cv. Langra as less susceptible. Khan and Khan (1960) and Singh et al. (1977b) considered Chausa as resistant. Likewise, the performance of the cv. Fajali against malformation is also replete with contradictory reports. Tripathi (1954) described Fajali as susceptible but Singh recorded it as resistant.

Kumar and Beniwal (1992) suggested that rating for the disease estimates should be based on pooled disease index over two consecutive years ('on' and 'off' years). Khan and Khan (1960) developed a method for disease rating. Kumar and Beniwal (1992) formulated rating formula for disease severity, disease incidence, and maximum disease incidence. Iqbal et al. (2004) recently assessed malformation of some commercially important cultivars in Pakistan following these formulae with some modifications. Both the authors categorized the mango cultivars according to their

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Table 7.1 Percent incidence of floral formation on different cultivars under different agro-climatic conditions

References	Location	Cultivar										
		Alphanso	Amrapali	Bhadau-ran	Bombay Green	Bombay Yellow	Bara-masi	Chausa	Dashe-hari	Fajali	Gulab Khas	Gopal bhog
Tripathi (1954)	Pantnagar, Uttarakhand				39.18	70.01		36.91		59.33		
Khan and Khan (1960)	Punjab, Pakistan	70–95						20–98	15–69			
Singh et al. (1961)	Saharanpur, U.P.					9.0						10.0
Chadha et al. (1979)	Lucknow, U.P.						20.0	60.0	84.0			
Kumar and Beniwal (1992)	Pantnagar, Uttarakhand							75.0	74.0			
Dhar et al. (1979)	U.P.						21.6					
Singh et al. (1979)	U.P.								24.80		36.62	
Badliya and Lakhanpal (1990)	Kangra valley, Himachal Pradesh	17.25	57.12		56.25							
Kumar and Chakrabarti (1998)	Faizabad, U.P.		60.0					19.0	45.20			
Wada et al. (2001)	New Delhi	37.68	43.77	1.1				52.83	31.50	32.60		
Ahmad et al. (2002)	Faisalabad, Pakistan							44.05	36.73			
Iqbal et al. (2004)	Faisalabad, Pakistan							18.6	26.83	24.6		
Mishra (2004)	Rewa, M.P.	30.37	27.48		1.53				30.98	19.45	3.79	2.49
Singh (2006)	Lucknow, U.P.	35.00							15.00			
Prakash et al. (2006)	Meerut, U.P.		33.33					21.66	25.45			
Gaur and Chakrabarti (2009)	BHU, Varanasi		50.3					21.16	38.13			

Table 7.1 (continued)

References	Location	Cultivar										
		Himsagar	Langra	Malda	Mallika	Neelum	Ratul	Ratana	Ramkela	Safeda	Totapuri	Zardalu
Tripathi (1954)	Pantnagar, Uttarakhand									32.12		
Khan and Khan (1960)	Punjab, Pakistan					2–8	45–50					
Singh et al. (1961)	Saharanpur, U.P.					Nil						
Chadha et al. (1979)	Lucknow, U.P.		72.0	68.0						95.0	60.0	
Kumar and Beniwal (1992)	Pantnagar, Uttarakhand				20.0	11.0						
Badliya and Lakhanpal (1990)	Kangra valley, Himachal Pradesh		9.37		55.0						16.53	
Kumar and Chakrabarti (1998)	Faizabad, U.P.	51.64	43.8		25.87	31.22						46.57
Singh et al. (1999)	Itanagar, Arunachal Pradesh						8.71			8.27		
Wada et al. (2001)	New Delhi	42.0	31.62		38.72	42.3	37.46	52.42			42.8	52.16
Ahmad et al. (2002)	Faisalabad, Pakistan		34.48	43.05			56.63					
Iqbal et al. (2004)	Faisalabad, Pakistan		15.92	25.13			31.02					
Mishra (2004)	Rewa, M.P.	21.68	1.61	17.15	29.05	3.06		52.86	12.87	2.03		20.69
Prakash et al. (2006)	Meerut, U.P.				24.55		5.0		9.9	10.55		
Gaur and Chakrabarti (2009)	BHU, Varanasi		17.56		35.50							

susceptibility on one to nine rating scale. The following formulas were suggested for calculation.

$$\text{Disease incidence} = \frac{N_1}{N_2}$$

where N_1 and N_2 represent the number of infected plants and total number of plants respectively.

$$\text{Disease severity} = \frac{D_1 + D_2}{T_1 + T_2} \times 100$$

D_1 and D_2, represent number of infected inflorescences per plant during first and second years respectively while T_1 and T_2 represent the corresponding number of total inflorescences per tree. The disease severity for the second year will be calculated similarly.

$$\text{Maximum disease incidence} = \text{Disease severity} \times \text{Disease incidence}$$

The disease rating scale is as follows: 1=free from disease (resistant); 3=0.1–1% disease index (DI) (moderately resistant); 5=1.1–10% DI (tolerant); 7=10.1–20% DI (moderately susceptible); and 9=>20% DI (susceptible).

Varieties from Southern India—where the disease incidence is sporadic—were found to be more severely affected when introduced into North (Mallik 1963; Singh and Jawanda 1961). Mallik (1961) reported that some of the varieties from Maharashtra viz. Alphanso, Pairi, Tharipady and Mundapa that did not have the disease in their native place were seriously infected when grown under north Indian (Bihar state) conditions. Similarly Singh et al. (1961) noticed that most of the mid and late season varieties are less affected. It seems that the mid and late season mango varieties or the cultivars grown under Maharashtra conditions escape infection due to higher prevailing temperature at the time of bud burst. Hence, it has been proposed that the disease resistance capacity of the cultivars may be further confirmed by artificial inoculation test and thereafter these may be included in the resistant breeding programmes (Mishra 2004). Thus, Zaccaro et al. (2004) tested the disease susceptibility of 15 cultivars by inoculating them with the fungus under controlled conditions and recorded significantly lower percentage of floral malformation as well as slow progression of the disease.

Prasad et al. (1965) screened 99 mono- and polyembryonic varieties of mango under field conditions and found all but one viz. Bhadauran to be susceptible to the disease to different extent from low to high. Bahadauran was proved a tolerant or resistant parent for breeding purposes. However, Wada et al. (2001) recorded manifestation of the symptoms on Bhadauran although the disease incidence was very low (1.1%). Recently one cultivar viz. Ellaichi from Lucknow (Misra et al. 2000; Singh 2006) and three cultivars viz. Langra Rampur, Malda Handle

and Asaugia Davban from Pantnagar, India (Kumar and Beniwal 1992) have been claimed not to be affected by the disease. Similarly, in Egypt, the cultivars Zebda and Hindi Anshas were reported to be rarely affected (Azzous et al. 1978). The malformation resistant capacity of Zebda was further confirmed by El-Ghandour et al. (1979) when they recorded that extracts of shoots or inflorescence of Zebda strongly retarded the growth of the *F. moniliforme* var. *subglutinans in vitro.* But no variety in Pakistan has been found to be free from the disease (Ali 1977). The important commercial cultivars like Keitt in Florida (Ploetz 1994), Handen in Mexico (Noriega-Cantu et al. 1999), Kent in Israel (Freeman et al. 1999) Tomy Atkins in Brazil (Sao-Jose et al. 2000) suffer serious yield losses due to this disease.

Chapter 8
Control Measures

The disease has been an enigma in its etiology, epidemiology and host-parasite interactions and it is no wonder that all kinds of methods have been tested from time to time. More significant ones are discussed under cultural management that include sanitation, water stress management, major and micronutrient management, chemical management including hormonal, acaricidal and fungicidal, anti-malformins, botanicals and biological management. These then were integrated to develop IPM and system management approach.

Cultural Practices

Sanitation

Narasimhan (1959) first attempted sanitation as a tool to control malformation. He conducted a trial on 20 mango trees which were heavily infected. The malformed panicles were excised 1–2 ft below the inflorescences and were burnt. The removal of malformed inflorescences completely freed 15 trees from malformation while five others developed only one or two malformed panicles. Similarly, Mallik (1963) reported success in controlling the disease by removing malformed panicles. Desai et al. (1962) confirmed the results of Narasimhan (1959) when he successfully controlled the disease up to 90% in the cultivar Rajapuri. He pruned the malformed shoots and panicles in July and August following the procedures of Narasimhan. The success of controlling the disease by pruning was reported by others also. Bindra and Bakhetia (1971) pruned malformed panicles 30 cm below the shoots during July on sucking type of mango plants and concluded that pruning may help in reducing the incidence even without applying any pesticides. Doval et al. (1976) pruned malformed panicles along with 30 cm of the shoots in July. The treatment reduced significantly the malformation in next flowering season. Singh et al. (1983) pruned shoots 22 cm below the malformed inflorescences during flowering season and found it reduced the incidence. Chib et al. (1986) observed that pruning and deblossoming, with or without fungicidal and acaricidal

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applications were successful in reducing floral malformation in cultivar Dashehari. Campbell and Marlatt (1986) and Darvas (1987) noticed pruning was highly effective and recommended the treatment for commercially adoption in south Asia. Later Manicom (1989) also confirmed pruning as a highly effective control measure against malformation. Pandey (2003) and Pandey and Chakrabarti (2004) in an elaborate study observed that total eradication or pruning of malformed panicles of yesteryear ('mother malformed panicle' that served as the source of fresh inoculum) or partial pruning leaving maximum six such malformed panicles on plants, reduced the disease incidence considerably and increased number of total panicles. But the reduction in number of mother malformed panicles vis-à-vis initial inoculum level, increased the rate of progress of the disease. Thus, the effect of pruning did not sustain long. It is presumed that increased number of buds in mango after pruning provided more infection sites and with availability of inoculum from neighbouring malformed plants the disease registered higher incidence. These observations corroborated with the earlier report of Berger (1988) who noticed that with availability of more susceptible tissues, the disease progresses faster. Hence, pruning only once does not seem to be an effective approach to keep the disease below economic threshold level. Kumar and Beniwal (1992) also recorded that pruning was quite effective in suppressing the disease for the first two years after pruning; but the plants produced malformed panicles again after two years. Thus, only systematic removal of diseased shoots and inflorescences for consecutive years may completely free the plantation from malformation (Narasimhan 1959; Mallik 1963). Initially plants receive very limited amount of inoculum from outward source and therefore rate of infection, is slow (Van der Plank 1960). After development of secondary source of propagules inside the plant, the infection rate increases and sanitation may not be of much help. Narasimhan (1954) controlled malformation by pruning plants that had only 28–30 malformed panicles/plant. But, Varma et al. (1971) failed to eliminate the disease even heading back of a plant as it was severely infected. The success seems to depend on the stage and extent of infection in the treated trees (Varma 1983). However, Saeed and Schlosser (1972) did not find any effect of removing of malformed inflorescences on the disease intensity.

Water Stress

Tahir et al. (2003) attempted to reduce the incidence of vegetative malformation by discouraging vegetative growth during rainy season by putting the plants under water stress. For this purpose, the leaching of water in the root zone was stopped using thick gauze polythene sheets. Drought stress discouraged vegetative growth during July and later months which reduced malformation because the flushes of this period were more susceptible. Development of vegetative buds was delayed and growth of floral buds was stimulated in response to water stress.

Scion Management

Khader et al. (1986) suggested to avoid removal of scion sticks frequently from young healthy trees for purposes of propagation to minimize the disease incidence.

Application of Nutrients to Control the Disease

Attempts were made to restore the normal health of malformed plants by applying farm-yard-manure (FYM) and balanced fertilizers or by spraying or injecting micronutrients.

Nitrogen Fertilizer

To maintain the normal ratio of C/N in the plant, application of nitrogen at higher disease levels have been recommended. Thus, Jagirdar and Shaik (1969) recorded that increased use of nitrogen reduced malformation considerably. Prasad et al. (1965) in a fertilizer trial observed that increasing level of nitrogen reduced the percentage of malformed panicles. Shawky et al. (1978a) sprayed urea (0.25, 0.5, 1.0, 1.5 and 2.0%) just before flower bud differentiation. This operation delayed time of flower bud opening. They recorded reduction of flowering malformation, increased number of perfect flowers and improved pollen grain viability. Best results were obtained with 1–2% urea. Similarly, Azzouz and Dahshan (1981) reported considerable reduction of flowering malformation with higher nitrogen rate alongwith Zn or Mn each at 0.3% applied in May and again in September. But Bindra and Bakhetia (1971) did not find any reduction in incidence of malformation with application of nitrogen even at very high doses. Cheema and Malhi (1986) in a field trial with cv. Dashehari applied nitrogen at 0–300 g/year of the tree age. The incidence of vegetative malformation (bunchy top) increased with rising nitrogen rates. The lowest incidence of floral malformation (45.8% compared with 80% in the untreated control) was observed with the medium nitrogen rate (200 g). Kishore and Syamal (2006) succeeded in increasing the percentage of ovary per panicle and normal anther both in malformed and healthy panicles by spraying urea (2%) in October.

Micronutrients

Tripathi (1955) reported that spraying of micronutrients ($MgSO_4$, $ZnSO_4$ and Borax) reduced vegetative malformation in Chausa. In Bombay Green, $CuSO_4$, $ZnSO_4$ and Borax reduced vegetative malformation by 7.67, 24.84 and 5.81% respectively. The

disease was reduced (5.11%) in control also. Thus, a significant disease reduction was recorded only with $ZnSO_4$. On the other hand, $MgSO_4$ increased the disease incidence. In the cv. Fajri, only $MgSO_4$ and Borax showed the disease reduction but the percentage of reduction was less than that of the control. The treatments failed to reduce the floral malformation in all the cultivars; rather disease incidence was increased. In case of trunk injection, when the microelements administered alone they decreased vegetative malformation. The lowering of the disease incidence was also noticed in control. Thus, appreciable control was noticed in treatments with Borax (12.85%), $ZnSO_4$ (6.36%) and $MgSO_4$ (9.10%). But such treatments had reverse effects on incidence of floral malformation. Trunk injection of all the elements combinedly increased vegetative malformation but decreased floral malformation by 5.13%. It was concluded that the disease was not caused by any deficiency. It appears lower micronutrient level is a result rather than the cause of the disease. Same experiments were repeated in 1961 by Singh et al. (1961) and they also confirmed the above observations. Like Tripathi (1955), El-Beltagy et al. (1979) applied a mixture of micronutrients, "Bayfolan", containing N, P, K, Fe, Cu, Mn, B, Zn, Co, and Mo at 100 ml/tree to root zone soil of 22-year-old trees of mango cv. Timour in February in an 'on' year. Bayfolan appreciably reduced the number of panicles, both total and malformed, but had no significant effect on number of healthy panicles and did not affect the percentage of malformed panicles. Similarly Saeed and Schlosser (1972) did not obtain positive results by removing malformed panicles and spraying the trace elements. Singh et al. (1961) gave foliar application of $ZnSO_4$, $FeSO_4$, $MnSO_4$ and $CuSO_4$ (0.2–0.4%) during first week of October but these did not substantially reduce floral malformation in Dashehari cultivar. But Minessy et al. (1971) succeeded to correct the deformity of 2.5 years old mango plants when Fe was applied @ 50 or 100 g/tree to the root zone soil in chelated form (containing 6–7% Fe). But the foliar application was ineffective. Similarly, Abo-El-Dahab (1977) could reduce vegetative malformation by application of metallic iron (6%) containing sequestrene. Peswani et al. (1979) recorded inhibition of floral malformation and reduction of fruit drop by applying K_2SO_4 to soil around the roots, plus a trunk injection of monocrotophos. Insecticide treatment alone was ineffective.

Prasad et al. (1965) did not find any direct relation between the dose of FYM and bunchy top stage of mango malformation. Hence, the contention that the disease increased with heavy manuring was not substantiated. Lowest level of both phosphorus and potash with high dosage of nitrogen (9-3-3), decreased the percentage of malformed panicles. Bindra and Bakhetia (1971) could not control the disease by applying NPK and thus concluded from their trials that malformation was not simply due to imbalanced NPK fertilization. On the contrary, Cheema and Malhi (1986) observed in field trials that bunchy top was reduced by high rates of P and K. But Kanwar and Kahlon (1987) observed that addition of phosphorus and potassium increased the malady while it was significantly reduced by applying nitrogen at high dose i.e. 300 g/tree. These experimental results did not help in devicing a fail-safe approach to reduce malformation through gross or subtle variations in nutrient regime or exogenous supply of micronutrients.

Hormonal Treatment

Growth hormones as potential control agents for malformation has been more closely investigated than any other group of chemical (Kumar et al. 1993). The effects of growth hormones, either separately or in combination with other measures, in reducing the incidence of malformation or in increasing the yield were investigated by several workers. But the results of different experiments were not consistent. The efficacy of different growth hormones tested alone was as follows.

Naphthyl Acetic Acid (NAA)

The observation that an imbalance of auxins and anti-auxins lead to the disorder prompted efforts in India to correct the imbalance by exogenous application of synthetic auxins (NAA 200 ppm) at flower bud differentiation as means of reducing floral malformation and improving yield.

Mallik et al. (1959) demonstrated for the first time that spraying of β-NAA over whole plant of the cv. Kalapady (introduced to Bihar from south) before flower bud onset improved the ratio of male and female flowers in treated (9:1) over the untreated control (20:1). Rawosh et al. (1983) reported that NAA even at 40 ppm could significantly reduce the fruit drop. Besides, NAA possesses chemotherapeutic activity against the *Fusarium* pathogen. Treatment with NAA and indole-3 acetic acid (IAA) reduced *Fusarium* wilt of tomato (Davis and Dimond 1953). Efficacy of plant growth regulators *in vitro* was relatively poor than fungi toxicants. Plant growth regulators reduce diseases by inducing changes in the host metabolism which regulate the growth of the parasite and/or the elaboration of toxins.

Majumder et al. (1970) treated malformed plants of the cvs. Chausa, Dashehari and Bombay Green with NAA (200 ppm) in the first week of October well ahead of flowering and observed about 78, 56 and 74% reduction in the incidence of floral malformation over the untreated controls in Chausa, Dashehari and Bombay Green respectively. Similarly when Planofix, a commercial product of NAA was sprayed at 200 ppm on Langra mango trees, the disease incidence was reduced (Shant 1975). Singh et al. (1977a) reported that NAA spraying led to 50% control of malformed panicles at 150 ppm. The sex ratio of staminate: hermaphrodite flowers was 5.8:1 in the control while 2.49:1 in the treated plants. In a 3-year trial Bajpai and Shukla (1978) recorded reduction in floral malformation in the cvs. Bombay Green and Bomaby Yellow by spraying NAA (150 and 250 ppm) in mid-October. The effect of treatments varied from year to year. Chadha et al. (1979) observed reduction of floral malformation by spraying Planofix (200 ppm) and NAA (200 ppm). However, Planofix showed better performance. Singh et al. (1983) observed reduction of malformation by 14% on 20 year-old trees with NAA (200 ppm). Singh and Dhillon (1986) reduced the incidence of floral malformation by spraying with NAA (200 ppm) prior to flower bud differentiation.

On the contrary, El-Beltagy et al. (1979) did not find any marked effects of NAA spray application on the incidence of malformation at 50 ppm (although total number of panicles increased). Bist and Ram (1985) reported that in cv Dashehari naphthalene acetic acid was least effective in controlling malformation under Pantnagar (Uttara Khand) conditions. Siddiqui et al. (1987) recorded that NAA gave over 94% control in Dashehari; but it was least effective in cv. S.B. Chausa.

Das et al. (1989) also reported that treatment with NAA was ineffective in controlling malformation incidence. Similarly Kumar and Beniwal (1992) failed to reduce the malformation by spraying NAA alone or in combination with fungicides.

Thus, the results provide inconclusive evidence in favour or against for application of NAA as management strategy for malformation of mangoes per se.

Gibberellic Acid (GA)

It has been observed that the incidence of floral malformation is higher in the panicles that emerge early. Hence, attempts were made to delay flowering with the help of GA and thus reduce the disease incidence and improve the yield. Singh et al. (1977) reported that GA treatment (150 ppm) increased the percentage of hermaphrodite flowers. The sex ratio of staminate: hermaphrodite flowers was recorded ca. 6:1 in the control while 2.6:1 in the treated plants. Shawky et al. (1978b) reported that GA spray (10, 25, 50, 75 ppm on Taimour) delayed flower bud opening, increased number of hermaphrodite flowers, fruit setting and reduced floral malformation. The best results were obtained with 50 ppm. Das et al. (1989) observed 50% reduction in malformation with GA_3 sprayed in October–November.

On the other hand El-Beltagy et al. (1979) reported that GA_3 at 100 and 250 ppm significantly reduced the number of total, healthy and malformed panicles and increased the percentage of malformed panicles. Kachru et al. (1972) treated buds of Dashehari in the 'on' year with GA_3 at 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} M in November–December. The highest concentration delayed bud break by over 2 months and 945 of the treated shoots produced vegetative shoots. Similarly Rawosh et al. (1983) observed inhibition of flowering when GA_3 (500–3,000 ppm) was sprayed in November–December.

Ethylene

El-Beltagy et al. (1979) reported that ethephon at 50 and 150 ppm increased the percentage of malformed panicles but slightly decreased that of healthy panicles. Rawosh et al. (1983) obtained highest percentage of flowering and fruits/panicles with six times spraying with ethephon (500 ppm) between September–November. Likewise, Singh and Dhillon (1986) observed that incidence of floral malformation was best reduced with concomitant increase in fruit yield by spraying with ethrel (ethephon, 500 ppm) at bud burst stage.

Mangiferin Metal Chelates

Attempt was made to develop an integrated disease management strategy in the background of the available information on etiology and epidemiology. The important principles of the strategy are (1) eradication of mangiferin inducer, (2) supply of micronutrients to the deficient plant parts with mangiferin metal chelates and (3) mangiferin based prophylactic spraying.

A low level persistent stress caused by the continued presence of infectious agent results in the accumulation of high level defense chemical compound (Kuc 1987) like mangiferin. Besides, in presence of the pathogen, micronutrients (zinc) or growth hormone (auxin) exogenously supplied to make up the deficiency promoted the disease (Ploetz and Prakash 1997; Rajan 1986) but in disinfected plants (either by pruning or by chemical treatment) flowering and fruiting instead are increased (Kumar and Chakrabarti 1998). Eradication of the stress factor(s) are essential to maintain the normal balance of mangiferin vis-à-vis other chemical components of the host plants.

It is stated earlier that due to accumulation of mangiferin at the infection site, transport system of the plant becomes disrupted. Thus, micronutrients which are carried by mangiferin due to its chelating property cannot reach the growing plant parts and thus normal growth is affected. When mangiferin metal chelates (Cu^{++}, Zn^{++}) (Fig. 8.1) are sprayed on the infected plants after proper pruning of the malformed plant parts, the compounds facilitate the supply of micronutrients to the

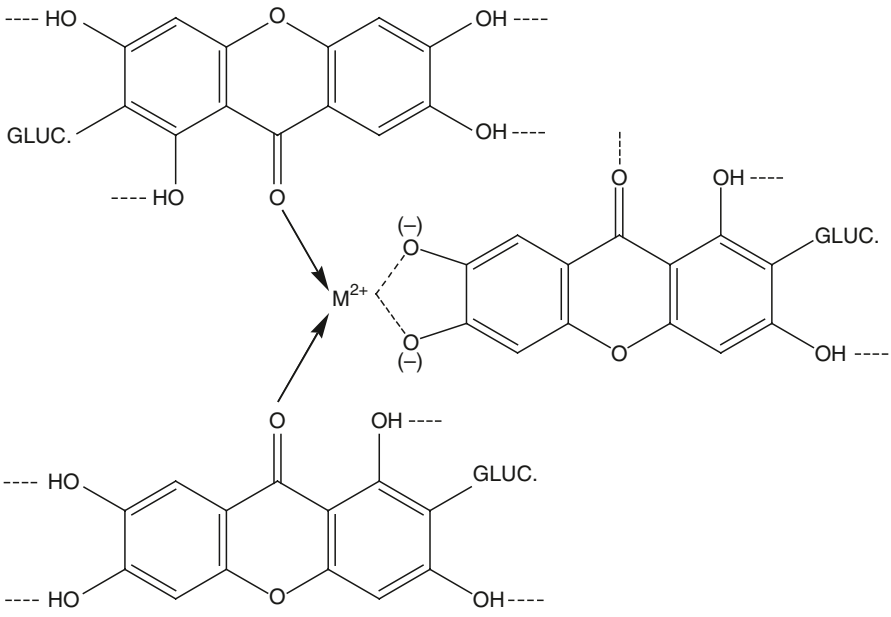

Fig. 8.1 Probable structure of mangiferin metal ion complex, where M is Zn or Cu

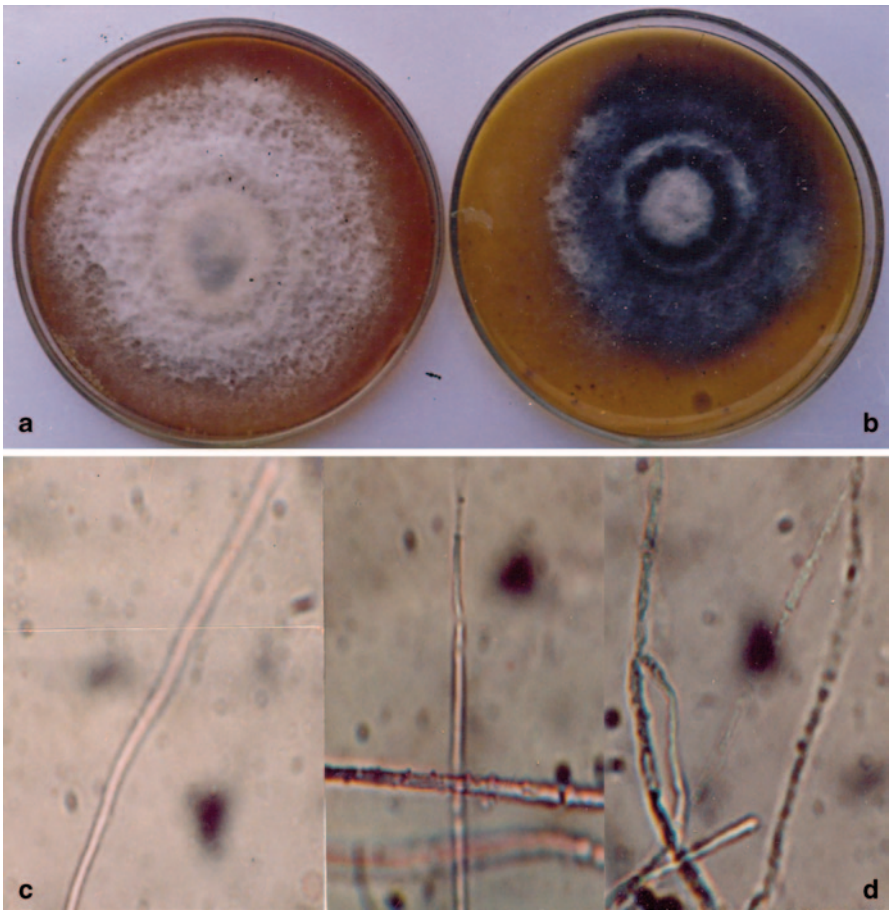

Fig. 8.2 Effect of mangiferin copper chelate on *F. moniliforme* var. *subglutinans*. *Grayish white* colony of *F. moniliforme* var. *subglutinans* on mangiferin copper chelate amended PDA (**a**), mycelia turned *black* after 5th day (**b**), hyphae in control (**c**), lysis and collapsing of hyphae from tip in the treated set (**d**)

deficient plant parts and normal growth of the plant is restored (Chakrabarti and Ghosal 1989; Kumar and Chakrabarti 1998; Chakrabarti et al. 2001; Ali et al. 2004).

The mechanism of mangiferin Cu^{++} chelates was also investigated (Gaur and Chakrabarti 2009). In mangiferin Cu^{++} chelate treated mycelia dry weight was initially increased over the control. But after 72 h. the mycelia turned black and disintegrated (Fig. 8.2b, d). Mangiferin Cu^{++} also inhibited conidia germination (Fig. 8.3c).

Along with eradication, prophylactic measures have to be adapted to keep newly developed buds safe from fresh infection. Once the chemical aberrations (accumulation of mangiferin and the *Fusarium* toxins) is complete, the spraying with anti-fusarial fungicides will not be effective (Summanwar 1967). Mangiferin copper chelate is better than the commercial preparations of copper fungicides.

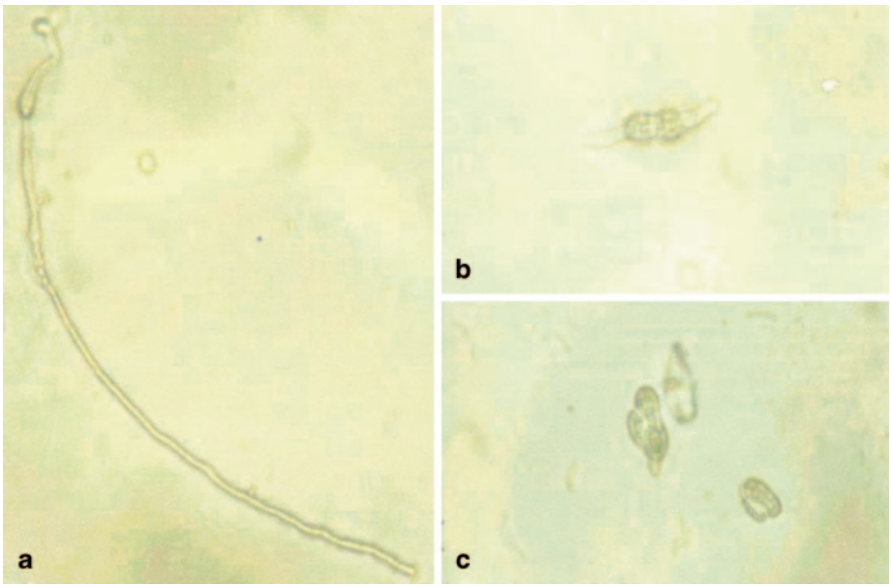

Fig. 8.3 Germination of conidia of *F. moniliforme* var. *subglutinans:* control (**a**), carbendazim (Bavistin) (**b**) and mangiferin copper chelate treated (**c**)

The conventional copper fungicides act only as contact fungicides. But copper ions complexed with carrier mangiferin is circulated throughout the plant system.

Mangiferin copper chelate not only eradicates the pathogen but due to its zinc sparing effects supply the host plant with zinc which are essential for growth and normal function of the plants. Mangiferin increases nitrogen content and reduces the disease incidence by normalizing C/N ratio. It decreases iron content that helps to control infection by creating iron deficient environment in host cells (Neilands and Leong 1986). Copper chelates stop influx of mangiferin into the developing buds. Mangiferin zinc chelates increases zinc, auxin and carbohydrate contents of the treated plants. Moreover, if the plants are pruned, the treatment increases flowering and fruiting (Kumar and Chakrabarti 1998).

Based on the above reports, it may be concluded that mangiferin plays an important role in determining the pathogenicity of the strain of *F. moniliforme* var. *subglutinans* and manifestation of the disease symptoms. However, by mobilizing accumulated mangiferin and preventing phytotoxin secretion the disease can be managed considerably. This, therefore, provides a promising tool as a possible and potent component of IPM.

Acaricide

Acaricides have been applied on mango plants to control malformation by three methods. Firstly, it is applied alone assuming that the bud mites are solely responsible for the disease. Secondly, the acaricides, based on the information that the mites

act as the vector, has been sprayed along with fungicides. Thirdly, it has been used as a component of an integrated disease management strategy presumably in recognition of the fact that other than the pathogen, the host and climatic factors also play important role in the disease development. Salient features of the considerable researches on effects of some acaricides in controlling mango malformation will be presented under several sub-heads.

Acaricides Applied Singly

Singh (1956, 1957) observed two sprays of diazinon (0.32%) at 15 days interval alone minimized the malformation. He (Singh 1962) also found Alboleneum very promising in reducing the malformed inflorescence. Desai et al. (1962) claimed that spraying with folidol or ekatin controlled malformation up to 99%. Nariani and Seth (1966) reported that fumigation of 1–2-years-old seedlings with methyl bromide (30–40 mg/l for 2 h) as prophylactic treatment could prevent the disorder. The intensity of malformation was significantly reduced by the following treatments in decreasing order of effectiveness: phosphomidon (0.3%), parathion (0.1%), thiometon (0.1%), methyl-demeton (0.1%) and wettable sulphur (0.25%) if sprayed from July to December at 21 days interval (Doval et al. 1977). Varma et al. (1971) observed that aphidan and diazinon (500 ppm) inactivated the *Fusarium in vitro.* Later Yadav and Varma (1969) found aphidan was very effective in killing mango bud mite also.

Yadav (1972) pruned 1 year-old malformed saplings containing mites leaving only single malformed bud and the pruned saplings were treated with 0.1% diazinon emulsion at monthly interval to keep the saplings mite-free. Because of the treatment, malformed buds developed normally. The incidence of floral malformation was also reduced significantly by pruning the malformed portions and then spraying with diazinon. The results were later confirmed by Rai and Singh (1967). Bindra and Bakhetia (1971) noticed that acaricides (dicrotophos or phorate) coupled with pruning of malformed tissues reduced the disease incidence effectively.

Contrarily, Prasad et al. (1965) reported that metasystox, endrin, diazinon, photex, kelthane and kerathane successfully inhibited the mite population on mango buds but failed to control the disease. Khan and Khan (1960) and Latif et al. (1961) also did not find acaricides to reduce the disease incidence. The acaricides, diazinon (Chadha et al. 1979) and metasystox (demeton-methyl) (Das et al. 1989) on the other hand were reported to be completely ineffective in controlling malformation.

Acaricides Applied in Combination with Fungicides

Summanwar (1967) reported that treatment consisting of pruning of malformed plant parts, followed by prophylactic spraying with a mixture of the fungicide (captan at 0.1%) and a miticide (akar 338 at 0.1%) and a sticker (Tenac) at 12 days

interval helped considerably to reduce the disease. Likewise, Khurana and Gupta (1973) recorded that pruning of malformed shoots followed by spraying with captan and diazinon gave satisfactory control. However, Bindra and Bakhetia (1971) reported that combined spraying with captan (0.1%) and phorate (0.2%) without pruning was not at all effective.

Acaricide Applied as a Component of IPM Strategy

Kumar and Chakrabarti (1998) developed an IPM technique in which phospomidon (0.05%) was applied on plants twice i.e. in May after harvesting of fruits and October (before onset of flower bud differentiation) (coincided with occurrence of high bud mite population) along with pruning of malformed shoots and panicles, application of fungicide (chelated copper), micronutrients (chelated zinc), and hormone (naphthalene acetic acid). The technique was tested successfully under large scale field trial.

Noriega-Cantu et al. (1999) used the acaricide, sulphur (3.6 g a.i./l), at monthly interval during vegetative period along with pruning, fungicides (captan and benomyl), potassium nitrate, general insecticide (malathion) and chicken manures. The total treatment resulted in slower rate of epidemic development and lower level of final disease incidence.

Recently Kumar and Chakrabarti (2007) recorded that prophylactic sprayings with monocrotophos, dimethoate and sulphur decreased the percentage of the fungal colonization of host cells by 17.05, 24.37 and 12.02% over control. Dimethoate was more effective in controlling malformation than monocrotophos.

Fungicides

When the species of *Fusarium* was reported to cause the disease in 1966, several scientists hastened to recommend systemic fungicides, usually successful against fusarial crop diseases, to control also the malformation (Varma et al. 1971). Of the systemic fungicides, efficacy of benomyl (Benlate), carbendazim (Bavistin) and thiophanate-methyl (Topsin-M) have been largely evaluated. Varma et al. (1971) reported that after treatment with benomyl and Aphidan, new healthy shoots developed from a bunch of malformed shootlets. Siddiqui et al. (1987) sprayed carbendazim thrice over panicles (4–6 cm in length) of cultivars Dashehari and Chausa at weekly interval. This reduced incidence of floral malformation by 95 and 91.3% in Dashehari and Chausa respectively. Iqbal et al. (1998) injected Topsin-M, benomyl and Folicur (tebuconazole) into trunk twice (September and January) out of which Benlate and Folicur registered 72.5 and 71.1% disease control respectively. Spraying of Benlate or Topsin-M (0.2%) in July has been reported to control the disease (Muhammad et al. 1999). Recently, Iqbal (2004) in a field trial first clipped

the malformed branches and then sprayed with Benlate (0.15%); the disease was reduced by 72.04%. Pandey (2003) and Pandey and Chakrabarti (2004) studied the effects of carbendazim (Bavistin) on *F. moniliforme* var. *subglutinans* both *in vitro* and *in vivo* and on disease development. It was observed that bavistin failed to stop the germination of conidia significantly. But the growth of germ tubes was severely affected (Fig. 8.3b). Bavistin caused lysis of the hyphae *in vivo* and reduced production of conidia. The rate of infection in treated plants was slowed down. However, it requires comparatively more time to bring down the disease incidence. In fact in the first year of the treatment, the disease incidence was recorded to be marginally higher in the treated plants as compared with the control. The disease controlling effects of bavistin were evident from the second year of the treatment.

But there are many reports that did not confirm the above positive observations. Ibrahim et al. (1975) reported that growth of *F. moniliforme* was inhibited *in vitro* by benomyl and captan (heterocylic nitrogenous compound); but these fungicides had no effect when sprayed on diseased trees. Chadha et al. (1979) tested a large number of fungicides viz. benlate (0.2%), captan (0.3%), difolatan (0.3%), demosan (0.1%), MBC (methyl 2-benzimidazole carmate) (0.2%), dithane M-45 (0.3%), karathane (0.1%), fytolan (0.3%), bavistin (0.1%) for two years. There were no significant differences between the control and treated plants in percent of malformed inflorescences. The systemic fungicides viz. benlate, bavistin and MBC rather increased the disease incidence. The combined application of captan and diazinon also increased floral malformation. Similarly, Diekman et al. (1982) did not find any effect on incidence of floral malformation after spraying benomyl and an acaricide (bromopropylate) together thrice i.e. in April, May and June. Sharma and Tiwari (1975) observed effective control of malformation with benomyl treatment. These results suggest that it did not work systemically inside the plant. Kumar et al. (1993) reported that soil drenching with carbendazim decreased the population of *Fusarium* in bunchy top affected tissues but failed to cure the diseased seedlings or suppress new infections. They also found that MBC was readily and rapidly absorbed by mango lignin and this might be responsible for the poor translocation of the fungicide within the plant. Recently Freeman (2007) also reported that fungicides within the plant do not move, but remain absorbed at the application site.

Other than systemic fungicides, captan and copper fungicides have been tested against the malformation. Summanwar (1967) reported that spraying of captan (0.1%) along with an acaricide (Akar) after pruning of malformed shoots as prophylactic spray was highly effective. This was confirmed by Khurana and Gupta (1973) who recorded satisfactory control after pruning malformed shoots and spraying with diazinon and captan. For propagation of mango through grafting, in a field trial, captan (0.2%) was sprayed over the scion shoots immediately after defoliation. The first spray was followed by another two sprays at 7 days interval i.e. 24 h before and 7 days after grafting. The treatment resulted in development of 80% healthy scion shoots with luxurious vegetative growth (Gaur and Chakrabarti 2009). The anti-fusarium effect of captan was mediated through enhanced production of mangiferin and increasing population of the antagonist, *A. niger*.

Chattopadhyay and Nandi (1977c) reported complete control of the disease with fytolan (copper oxychloride) at 0.02% applied once before inoculation followed by two other sprays at interval of 5 and 8 days. Gafar et al. (1979) also found copper oxychloride (0.25%) and dithane M-45 (mancozeb) (0.25%) were effective in reducing the disease. Bindra and Bakhetia (1971) observed that captan alone or in combination with phorate without pruning were ineffective in reducing the disease. However, Das et al. (1989) did not notice any positive response of Blitox (copper oxychloride) treatment.

Anti-malformins

The activity of anti-metabolite compound, malformin, is reported to be inhibited by thiol compounds or sulfhydril reagents (Suda and Curtis 1964). Recent investigations on growth substance imbalance with particular reference to malformins has prompted the use of anti-malformins to counteract malformin response generated in the panicles. Ram and Bist (1984, 1986) applied glutathione (2,240 ppm) and ascorbic acid (2,110 ppm) over the 4–6 cm long mango panicles and reported that the malformed panicles turned healthy after 15 days of application. They recommended three sprays of the compounds after appearance of malformation. Siddiqui et al. (1987) confirmed that potassium metabisulphite was very effective in reducing development of floral malformation in the cultivars Dashehari and Chausa. However ascorbic acid was comparatively less effective. Singh and Dhillon (1989a, 1990a), and Bist and Ram (1990) claimed control of floral malformation with spraying of glutathione (560 ppm), silver nitrate (2,400 ppm), ascorbic acid (1,055 ppm) and potassium metabisulphite (560 ppm). However, Kumar and Beniwal (1992) and Rajan (1986) did not find reversal of the floral buds destined to be malformed into normal ones after the above treatments. Kishore and Syamal (2006) also did not find anti-malformins to improve fertility status (normally developed ovaries and anthers) either of healthy or malformed florets.

Botanicals

Leaf extracts of *Ruellia tuberosa* L. applied at the time of flower bud differentiation in October significantly reduced the floral malformation in cv. Dashehari (Pandey 1996). The reduction was found to be associated with an increase in IAA and polyphenoloxidase activity and total phenol contents in the bud. Ghosal et al. (1978a) also reported potentiality of extractives of *R. tuberosa* as foliar fungicide against *F. oxysporum* f. sp. *carthami,* the causal agent of safflower wilt. They identified presence of three phenolic compounds viz. 2, 6-dimethoxyquinone, acacetin and a C_{16}-quinone in *R. tuberosa.* The extractives of the plant inhibited the growth of *F. oxysporum* f. sp. *carthami,* and its spraying declined the incidence of wilt of safflower plants although growing in sick soil of *F. oxysporum* f. sp. *carthami.*

Bioagents

Aspergillus niger van Tiegh has been reported to be a constituent of natural mycoflora of malformed panicles and shoots particularly when they dry up and undergo rotting after rains (Dam 1992). He also recorded that with increase in population of *A. niger* the population of *F. moniliforme* var. *subglutinans* declined. Ali (1980) isolated *A. niger* along with *F. moniliforme* var. *subglutinans* and observed antagonistic activity against the *Fusarium.* Rath et al. (1978) reported the presence of *A. niger* on malformed shoots. Noriega-Cantu et al. (1999) also observed frequent presence of *A. niger* over malformed panicles with *F. moniliforme* var. *subglutinans.* Pandey (2003), and Pandey and Chakrabarti (2004) explored the potentiality of *A. niger* to be used for management of malformation. In *in vitro* study, *A. niger* was found to overgrow *F. moniliforme* var. *subglutinans* (Fig. 8.4a) and finally to parasitize the latter (Fig. 8.4b). In a field trial, spore suspension of *A. niger* (662 conidia/ml) was sprayed in hot humid evening of September over necrotic malformed panicles on which growth of *F. moniliforme* var. *subglutinans* were evident. After 15 days of

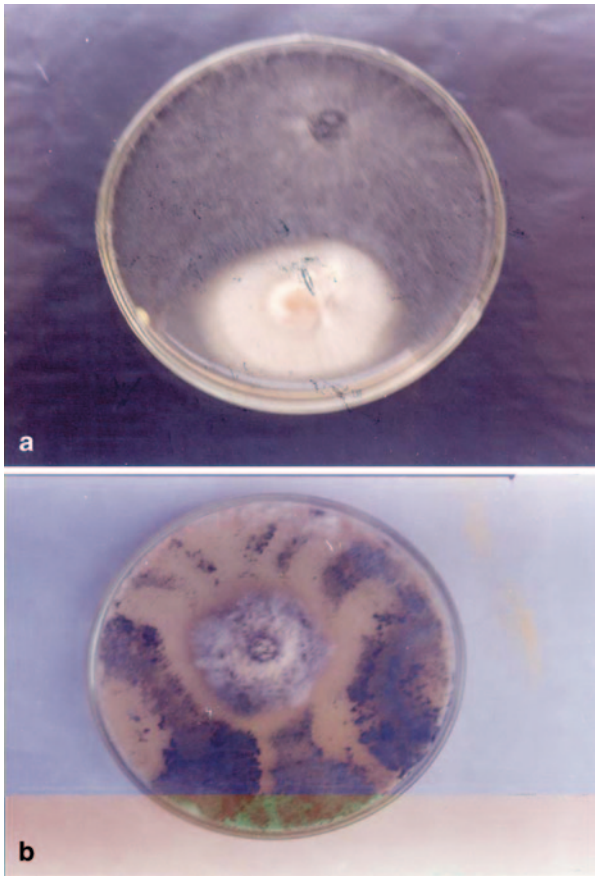

Fig. 8.4 Inhibition of growth (**a**) and parasitization (**b**) of *F. moniliforme* var. *subglutinans* by *Aspergillus niger*

spraying the population of the *Fusarium* over treated and control plants were counted. The estimated population of the *Fusarium* on treated and control malformed rotten panicles were 54 and 336 c.f.u./g respectively.

The results with botanicals and bioagents have not reached a concrete information level by which such steps can be incorporated into a viable IPM.

Integrated Management

Principles of Management Strategy

Lack of information on physiology of pathogenesis and epidemiology of the disease had been a limiting factor to establish a rational management strategy. Recently an epidemiological descriptor of mango malformation has been published (Chakrabarti and Kumar 2000). According to the descriptor, the disease is polycylic and the pathogen, *F. moniliforme* var. *subglutinans,* is polyetic and host specific. Maximum fungal population is recorded during February–March while the highest disease incidence in July–November. Latent period extended from late November to early February. New crops of conidia (propagules) on host surface were formed during July to September. The disease was transmitted by vector (mites) and infected scions. The gradient of spread within a tree canopy is steep. The plant to plant infection was slow. Logarithmic phase started at a low percent (1.34–5.01) of disease incidence. Mean maximum disease incidence in regular and alternate bearers was 40–48 and 72–73% respectively. Pattern of epidemic in former one was sigmoid while in the latter it was bimodal. Duration of the epidemic was year round. Thus, rate of plant to plant dissemination was slow and propagules for dissemination were available for a short period. But tissues once infected remained viably infected for significant amount of time, thus providing the necessary small amounts of inoculums that is required to start a fresh epidemic under favourable environment. Therefore, rate of increase of the disease could be minimized through sanitation (removal of malformed shoots and panicles and killing of propagules by chemical or biofungicides). Freeman (2007) reported that the pathogen remained viable in various parts of the tree for up to 7 years indicating that it survives in woody portions of the tree where lateral buds were present. The latency period (time between inoculation and symptom production) of the pathogen ranged from 40 to more than 200 days.

In nature, the amount of initial inoculum is controlled by vertical resistance. Since no species of mango is known to possess vertical resistance against the pathogen, the control over initial inoculum level could be achieved with the help of sanitation. However, the alternate bearing mango cultivars due to their ‘off year’ phenomenon provide vertical resistance-like advantage more frequently (Chakrabarti et al. 2005). Therefore, in the epidemic prone area like northern India, the orchardists should grow alternate bearing cultivars with maximum genetic heterogenecity in the orchards. The plant to plant spread of the disease in an orchard having mixed cultivars of regular and alternate bearers was slower than orchards with monoblock

cultivation of hybrids e.g. high density orchards of cultivar Amrapali (Kumar and Chakrabarti 1997b).

The pathogen multiplies primarily over dead rotten malformed mango panicles and shoots immediately after rainy season. Hence, the malformed panicles should be removed before onset of rains, preferably after harvesting of fruits. During spring and autumn flushes, the buds are highly vulnerable to fresh infection. Hence, at this time the newly emerged buds should be protected with broad based fungicides that is translocated from site of application to other parts of the plant. The copper fungicide (e.g. copper oxychloride) or captan or mangiferin copper chelates have been found to be very suitable. Phosphomidon was more effective with mangiferin copper chelates (Chakrabarti et al. 2001). To protect the tender buds from injuries inflicted by vectors (mites) application of acaricides (phosphomidon or dimethoate) is suggested simultaneously. Besides, spraying with NAA, zinc ions in the form of mangiferin chelates or amino acid based metal chelates (Chakrabarti et al. 2006) and urea at the time of flower bud differentiation or growth of the panicles will replenish the depleted amounts of these essential compounds in the plants long suffering from malformation. A combination of eradication of malformed panicles and shoots and spraying with metal chelates considerably reduced the fungal population and floral malformation and concomitantly increased the yield (Chand et al. 2002). Intensive control measures should be taken when the plants are in between 5–25 years old, the most susceptible stage for both vegetative and floral malformations.

Chand et al. (2002) tested three chelating agents viz. mangiferin, ethylenediamine tetrahydroxyacetic acid (EDTA) and amino acid for their micronutrient mobilizing capacity in *M. indica*. Mangiferin, being a natural metabolite of mango, served as the best chelating agent followed by amino acid and EDTA. Amount of copper and zinc ions in buds of trees from which malformed shoots and panicles were not removed was more after micronutrient treatment but its consumption in comparison with buds of pruned trees was less. The amino acid based metal chelates (Aminocel Gold, Excel Crop Care Group, India) containing some amino acid components like alanine, glycine and metal ions viz. copper (0.3%) and zinc (0.2%) ions seem to induce autoimmunity in mango plants (Chakrabarti et al. 2006). Aminocel Gold was found to increase 3-indole acetic acid, mangiferin content and polyphenol oxidase activity while the activity of catalase, the free radical scavenger and suppressor of host defense mechanism, was reduced.

Recommended Management Strategy

The IPM strategy proposed by Kumar and Chakrabarti (1998) (Fig. 8.5) included the following treatments: eradication of malformed shoots and panicles after spring and autumn flushes (May and October), spraying with acaricide (phosphomidon or dimethoate 0.05%) immediate after emergence of new buds (February, May and October), spraying with chelated copper (40 ppm) (mangiferin chelate or amino acid based chelate or copper oxychloride or captan 0.2%) (August–September and

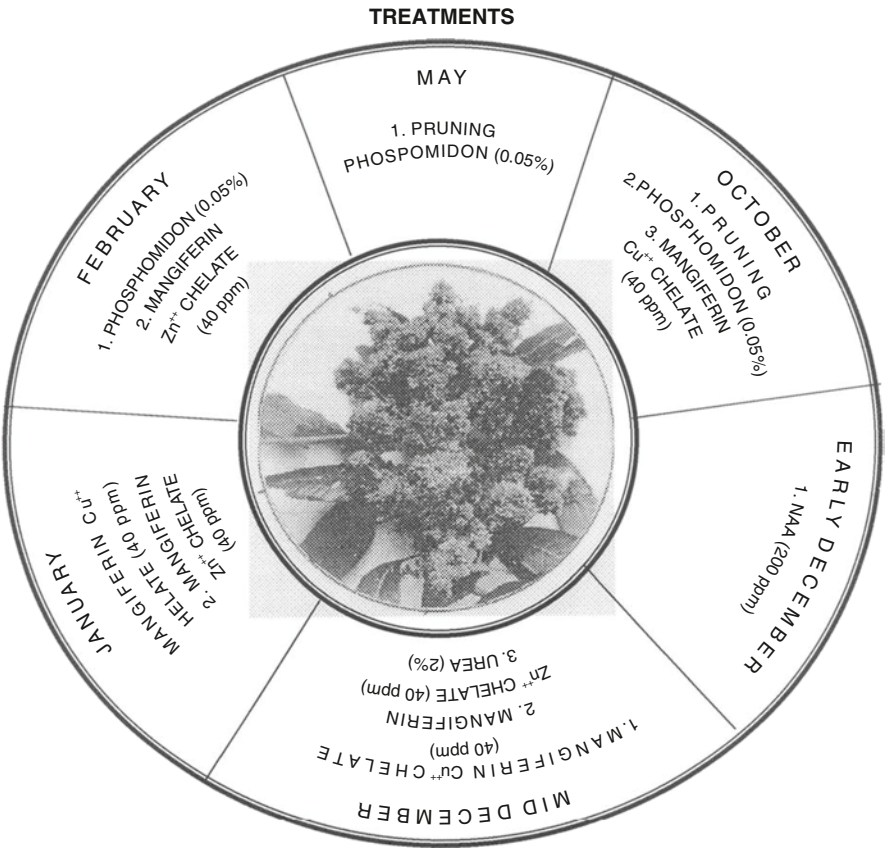

Fig. 8.5 Integrated management strategy for malformation

December–January) immediately after emergence of vegetative and floral buds, spraying with chelated zinc twice (40 ppm) (December and February) after emergence of flower buds and at developing stage of panicles, naphthalene acetic acid (200 ppm) once (early December) prior to flower bud initiation and urea (2%) (early December). To check powdery mildew and hopper sulphur fungicide and insecticide (monocrtophos) were also sprayed. The treatments for consecutive two years of 500 plants belonging to different cultivars in Rudauli-Sohawal mango belt of Uttar Pradesh, India reduced the disease by 26% with a concomitant increase in fruit yield by 32%.

The integrated management strategy of Noiega-Cantu et al. (1999) consists of removing of diseased shoots (80 cm below the lowest diseased shoots), four copper fungicide (copper oxychloride at 2.6 g a.i./l) and five acaricide (sulphur 53.6 g a.i./l) sprays at monthly intervals during the vegetative period followed by three sprays of fungicides (captan at 1.5 g a.i./l, benomyl at 0.25 g a.i./l, and mancozeb at 4 g a.i./l) in succession at fortnightly intervals from before flowering until set fruit. Besides,

the management strategy includes control of ants, spraying of potassium nitrate (3%) over the whole canopy to promote uniform flowering and one general insecticide spray (malathion at 1.5 ml a.i./l) and addition of chicken manures (2.5 kg/tree once a year). The treatment resulted in slower rate of the epidemic development, lower level of initial and final disease, and lesser areas under the disease progress curves with subsequent increase in fruit yield and the benefit-cost ratio. Thakur et al. (2000) reported considerable reduction in both vegetative and floral malformation by treating the plants with the combination of rogor (2 ml), multiplex (3 ml) and urea (40 g) in 1 l of water. Lopez-Estrada et al. (2005) developed one integrated management strategy which was very effective in the agro-climatic conditions of Mexico. They suggested pruning of malformed shoots and panicles after harvesting of fruits and at the time of vegetative growth and blooming, application of organic manures and chemical fertilizers (macro- and micronutrients) during vegetative (June–September) and panicle (December and February) growth, foliar application of nitrogen before flowering (November), enforcing water stress before flowering (October–November) and application of broad based fungicide (January) and insecticide (March–May, time for increased insect activity including mites).

Quarantine

To protect Queensland's 80 million $ mango industry from a new threat i.e. mango malformation disease, the government of Australia has promulgated Plant Protection (Mango Malformation Disease) Quarantine Act, 2008 under the Plant Protection Act 1989 (Anonymous 2008). Under this act, the whole Queensland is declared as a pest quarantine area for mango malformation. The objectives of this act are: (1) to prevent introduction of mango malformation disease in the pest quarantine area, (2) to prevent or control the spread of mango malformation in the pest quarantine area and (3) to control or remove mango malformation in or from the pest quarantine area.

Epilogue

The Problem

Mango (*Mangifera indica*) finds multifarious use of its different plant parts since ages though the most precious consumable is its fruits, that though varying from variety to variety, is very sweet and tasty to the palate. It is this use, other than its use in Hindu rites, that made it a well known tree back from the time of Buddha or even earlier. Mango most probably originated somewhere in S.E.Asia including Burmah (Myanmar) where several species grow widely. Mango saplings moved to and from the seat of origin not only to the countries of that region but also as far as Celon (now Sri Lanka), India, Mauritius etc.—all parts of greater India that existed notionally well before the birth of Christ. The plant received little attention from the Western scientists in the early times as it was restricted to tropical and sub-tropical regions in the far east. Subsequently it spread to middle-east including Israel, Africa from Portuguese colonies in India and thence to Brazil, West Indies and Florida in USA. Introduction to California and Southern China took place subsequently. These were mostly the polyembryonic varieties having lower pulp:stone ratio and not so tasty.

The monoembryonic varieties developed largely during the period of Moghuls under the patronage of King Akbar and subsequently the Nawabs of United Provinces (now Uttar Pradesh) in north India around its capital Lucknow (Malihabad and Kakori in particular) and Nawab of Murshidabad in Bengal. These varieties, often monoembryonic developed mostly by veneer grafting, were much more susceptible to various pests and diseases of complex etiology.

One such disease of complex etiology, mango malformation, initially noted in aged and poorly managed plantations, seriously gained in severity as a function of time and caused severe crop losses and sometimes led to discarding the severely affected plantations. Although many other pests of mango, particularly the severe ones, have now seen the light of management through integration of several options, the malformation disease remained unmanageable—so much so that mango saplings and veneers were not acceptable anywhere outside India. The prime varieties of U.P. therefore do not find a place in other countries even when having similar

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agro-ecosystem characteristics, In different biomes or eco-zones, these varieties when grown do not provide the same yield and flavour characteristics.

These observations make it more imperative that techniques of clonal propagation of disease-free planting material through tissue or similar culture be standardized so that the best varieties can be grown safely in similar eco-zones without fear of malformation and loss of yield and flavor characteristics.

Further, gene sequencing of varieties and clones is essential in relation to malformation susceptible and resistant plants. At present only chromosomal anomalies in abortive pollens of malformed panicles have been investigated. Such anomalies in malformed shoots and panicles are totally lacking. Such investigations will probably clarify to a large extent why one finds so much difference in symptoms of malformation depending upon the agro-climatic zone in which these plants are raised and may also help in identification of sequences responsible for susceptibility and resistance. Biotechnology tools used intensively will help explain the different types of symptom expression-both vegetative and floral malformation in UP; only vegetative malformation in most parts of Bengal and Kerala; compact bunchy top in var. Amrapali or tiny malformed panicles in the resistant variety Elaichi.

Etiology

As is the case for most disease of complex etiology in perennial trees, a large number of biotic and abiotic stresses have been identified to be responsible for the causation of the disease from time to time. The inflorescence malformation was noticed in 1891 and the vegetative malformation in 1951 and both were referred to as bunchy top. The name mango malformation was coined by Tripathi (1954).

A close association with an eriophyid mite, *Aceria mangiferae* led to this being identified as the possible cause of the problem but conclusive proof for causation could not be adduced over the years.

A considerable volume of experimental results were next generated to prove these symptoms of malformation to be caused through one or other physiological disturbance(s). These include nutrients that in turn included N, P, K, Zn, Cu to name a few along with the identified fungal pathogen. *F. monilifore* var. *subglutinans*. The deficiency itself resulted not from a chronic deficiency of such nutrients but through an electrolyte loss in cells of malformed leaflets. Other ascribed it to a disturbance of C/N ratio but data generated once again by different workers were contradictory and unconvincing.

A considerable body of data was generated to show that hormonal imbalance occurs in malformed shoots and panicles and at another point of time malformation was thought to be caused by an hormonal imbalance that could be auxins, gibberellic acid derivatives, cytokinins, ethylene or abscisic acid. Other abiotic factors

thought to have contributed to malformation included phenols, phytosterols, nucleic acids, amino acids, proteins, chlorophyll or even enzymes and toxins.

None of these abiotic factors could produce the disease through various manipulations. Meanwhile a case for biotic origin was gaining ground.

Other than mites, S. P. Raychaudhuri and his team made intensive studies on the management of this disorder on the premise that it caused by some mycoplasma-like organisms. As the premise was not based on experimental findings, the management also ultimately was doomed to failure. Some amelioration of malformation reported on application of tetracycline could be due to its effects on the associated microbiota. Similarly, efforts to prove the disorder to have a viral origin also was not convincingly demonstrated. Grafting or budding of susceptible tissues led to erratic transfer of malformed characteristics in the grafts. Sap inoculation also was not invariably successful. Veneer grafting also did not transfer the malformation characteristics in the healthy parts.

There is now a convincing body of evidence to suggest that primary causative agent for mango malformation is the fungal pathogen, *F. moniliforme* var. *subglutinans* which seems to be invariably associated with all the various expressions of malformation. Chakrabarti and Kumar (1998) adduced evidence to show that this species is a special form of identified as *F. moniliforme* var. *subglutinans* f. sp. *mangiferae*. Britz et al. (2002) produced arguments for malformation causing *Fusarium,* section *Liseola* belong to *F. mangiferae*. The Chinese isolates were identified as *F. proliferatum*.

This pathogenic fungus' physiology was investigated in details *in vitro* and it was shown to be able to utilize a large variety of C and N sources and was able to grow over a temperature range of 10–40°C with 30°C being the optimum.

From time to time many other fungal species have been claimed to have been associated with mango malformation without being able to adduce sufficient evidence for such association. Chakrabarti and his team adduced convincing evidence that *in vivo* growth of the pathogen in mango tissues internally was modulated by the host metabolites and that on the plant surface by the environmental parameters.

Host-Pathogen Interactions

There is sufficient experimental evidence to show that the fungal pathogen requires an exogenous wound or possibly a vector to facilitate host infection. increasing evidence is now accumulating to show that the mite *A. mangiferae* could be the primary vector for this pathogen. This however, did not eliminate the possibility of direct penetration through micro-wounds caused by such events as lashing rains, hailstorms, birds etc. The most susceptible site for penetration was possibly the bud meristematic region.

The fungus produces the usual battery of cell wall degrading enzymes though C_x activity was weak. There is also convincing evidence that the host defense mechanisms largely inactivate these hydrolytic enzymes.

Following invasion of the apical buds by the fungus, β-1, 3, glucanase increases and this was shown to induce increased production of mangiferin. The mangiferin accumulated in the cortical cells surrounding the fungal infection. The abundance of mangiferin restricted the further colonization of host tissues, preventing the pathogen to find ingress into otherwise dead xylem tissues, thus preventing systemic infection. The excess mangiferin also inhibited CWDE activity that in turn restricts the pathogen around the site of infection.

Mangiferin accumulation probably is the key to the host specificity of the pathogen *F. oxysporum* f. sp. *conglutinans,* specifically the one now designated *F. mangiferae* (Gamliet-Atinsky et al. 2010) and *F. proliferatum* (Zhan et al. 2010). *F. mangiferae* is a strain of *Fusarium* that did not produce fusaric acid *in vivo* and *in vitro*. However, it may be safely concluded that higher tolerance to mangiferin enables to enable this f. sp. to be pathogenic while other f. sp. of *moniliforme* fail on account of this being restricted/occluded by the presence in the cells of higher dosages of mangiferin.

The weight of evidence in favour of the hypothesis that defines why infection is restricted to the inflorescence or vegetatively growing regions through a modulation of the phenolic mangiferin in surrounding tissues. However, further in depth investigations are needed into role of mangiferin in determining the impact of this pathogen on the host including its restriction to apparently nutrient-rich tissues of the inflorescence or the vegetative apical buds.

Epidemiology

The investigations into the climate, weather and host variables and their interactions, bearing habit and time of flowering were investigated in relation to varieties, and then quantified to trace the patterns of epidemic both temporally and spatially. The data so generated was subjected to mathematical modeling using logistic, sigmoid, Weibull and other functions. Rajan and Majumder (1995) and Noriega-Cantu et al. (1999) found Gompertz model to give best fit for describing the epidemic. Pandey (2003), on the other hand, fitted temporal progression to linear, quadratic and cubic curves finding later to give the best fit.

These studies also led to the development of prediction equations based on weather, host and pathogen variables and their control varieties.

Some information regarding mechanism of natural resistance in mango plants against malformation that includes escaping of the disease by producing late in the season i.e. during warmer period, reduction of initial inoculum through alternate bearing phenomenon and killing of the pathogen with the help of its natural antagonists like mycophagus mites or *Aspergillus niger* etc are made available in the present volume. But it seems there may be more beyond the available information. Detailed knowledge in this direction may help to design an eco-friendly management strategy.

Management

Like any other disease where etiological complexity persists for long periods of time, all kinds of management strategy are tested at some or other—many eliciting a low level of possibilities while some were found to be meaningful.

Among the cultural practices of promise, pruning (sanitation) the malformed inflorescences and vegetative buds proved to be very effective, as malformation intensity was invariably reduced in the year subsequent to such operation. However, pruning the mother malformed panicles often caused proliferation of disease through increase in potential infection sites that invariably resulted when pruning operation was undertaken. Thus, single pruning operation invariably increased malformation in later years. Pruning was cumbersome and likely to succeed when practiced rigorously for several years.

Erratic results were obtained in various experiments through creating water stress in rainy season, application of major and micronutrients, application of hormones specially NAA, GA and ethylene.

These results showed that any strategy will have to be combined with pruning in order to be meaningful and sustainable. On the other hand it has been hypothesized that accumulation of the defense product mangiferin needs to be diluted as it chelates Cu^{++} and Zn^{++} ions making them unavailable in the growing regions (normal growth was resumed when plants were sprayed with mangiferin metal chelates after removal of malformed plant parts).

Since mites are held responsible largely for vectoring the pathogen into the host, mite management through use of acaricides like diazinon, phosphomidon, thimeton, aphidan, metasystox, karathan etc. and some success was invariably reported. Combining acaricides with fungicides gave even better results. The fungicides tested singly or in combination with acaricide, included carbendazim, benlate, tebuconazole produced results that were sometimes positive and sometimes negative leading to no conclusive evidence.

Similarly, a lot of experimentation have gone into the management using antimalformins like ascorbic acid or glutathione (to counter the malformation response generated in the panicles), botanicals and also bioagents like *Aspergillus niger*. They have largely remained inconclusive and experimental curious. However, several options being shown to be of promise, the stage appeared to be set by the end of the last century for developing IPM strategies for management of mango malformation as also for expert system for optimizing mango yield that could then be linked the Global Positioning System (GIS) through Global Information System (GIS).

Mango cultivars have been categorized as resistant or susceptible on the basis of their performance against the malformation disease under field conditions. The disease screening under natural conditions often yielded conflicting results. Therefore, disease resistant capacity needs to be confirmed by artificial inoculation of the host plants. The development of improved inoculation techniques seems to have made the task easier.

Inspite of the nature of the disease, laws of quarantine have not been implemented in any country other than Australia and recently in South Africa (de Graaf 2010). Had the quarantine laws been strictly imposed when malformation was first noticed in Bihar in 1891, the disease could not have spread so far and wide in India. Almost simultaneously, however, spread of wart of potato for Darjeeling, West Bengal in India was restricted by the same quarantine laws.

IPM Strategy and Expert System

Finally, an expert system for optimizing the plantation yield in general and malformation management in particular has been developed and is being constantly fine tuned (Chakrabarti and Chakraborty 2006, 2007). The software so devised, on limited testing, shows that it enhances the performance of the farmers, extension personnel, reduces time required to resolve the problem, making mango cultivation both more efficient and more profitable.

In the last decade, a detailed epidemiological descriptor (Chakrabarti and Kumar 2000) of mango malformation has been made use of in devising a stepwise, sequential management protocol for this problem. The steps recommended are:

- Meticulous removal of malformed panicles from affected mango trees before the advent of rains.
- Protection of buds in spring and autumn flushes by spray application of broad spectrum fungicides like captan, copper oxychloride. Chelated copper application was a good alternative.
- Bud damage by mites that also may serve as vectors for the pathogen may be restricted through simultaneous application of acaricides like phosphomidon or/dimethoate.
- Application of mangiferin chelates and urea at the time of flowering to replenish nutrient supply affected through infection.
- Need-based management of powdery mildews and/or hoppers by application of suitable/recommended pesticides.
- The strategy has boosted the yield of mangoes by nearly 32%.

A similar strategy was developed in Mexico (Noriega-Cantu et al. 1999) that consisted of pruning of affected shoots, followed by four fortnightly copper based fungicide applications, five fortnightly application of acaricides and three sprays of fungicide for the pathogen. Beside, a spray of 3% KNO_3 was recommended for uniform flowering.

This epilogue to a detailed narration in the book of the issues and achievements in understanding mango malformation, a dreaded disease causing havoc to the crop globally, hopefully helps in gauging the present day understanding of the problem and for identifying the key thrust areas where more intensive research inputs are needed. These include, among others:

- Sequencing the pathogenic strains of *Fusarium mangiferae,* the primary causative agent of this problem.

- Pinpointing the role of mangiferin in the expression of the disease.
- Use of tissue culture and other biotechnological tools for production of disease-free clonal material.
- Identifying more target specific toxophores for mite and pathogen management, requiring far less number of applications.
- Knowledge-based improvement of the existing Expert system and its ready availability to growers and extension workers.

It is to be hoped that the next decade will see a far more efficient management system for the dreaded mango malformation disease that scourge the mango orchards today.

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