Fruit and Vegetable Diseases

Edited by K.G. Mukerji

FRUIT AND VEGETABLE DISEASES

Disease Management of Fruits and Vegetables

VOLUME 1

Series Editor:

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FRUIT AND VEGETABLE DISEASES

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Created in the United States of America

SERIES EDITOR'S PREFACE

The advances made in food production may be lost if adequate attention is not given to plant diseases due to pathogens, pests and physiological disbalance. Introduction of new cultivars have resulted in addition to already existing major diseases, certain minor and new diseases and have assumed serious proportions resulting in considerable damage to the crop. Sometimes even epiphytotics have occurred resulting in total loss of the crop.

A recent survey indicates that the world population has increased by 90% in the past 40 years while food production has increased by only 25% per head. With an additional 15 billion mouths to feed by 2020, farmers worldwide will have to produce 39% more. Seeing the load of food requirements the eating habits are slightly changing and shifting towards fruits and vegetables.

Fruits and vegetables make a unique contribution to human diet as the key source of nutrition for around 6 billion ingredients for healthier nutrition in modern technological societies.

During the past twentieth century, pant pathology has witnessed a dramatic advancement in management of plant diseases through in depth investigations of host parasite interactions, integration of new concepts, principles and approaches. Effort is being made to bring out this book in several volumes to compile the achievements of twentieth century with regards to disease managements of fruits and vegetables which otherwise is widely dispersed in various scientific journals, government reports and university dissertations *etc.* and to develop future strategies for the new millennium.

Disease Management means use of a combination of methods to check a whole spectrum of pathogens, pests, physiological changes within a particular cropping system. The aim of this series is to provide an overview of Disease management of fruits and vegetables, highlighting the major problem areas and contentious issues and where possible attempting to identify promising lines and directions for future research and implementation. Disease management involves a number of stake holders ranging from scientists to farmers and agribusiness to consumers. As a reference book series for students', researchers, managers and administrators, emphases is placed on the underlying principles and experimental approaches to the science that underpins

the development of working management systems. Disease management is a holistic science rather than emphasis on isolated disciplines. For it is still at this holistic level that the greatest and most exciting advances are to be made.

For this reason we decided to publish several volumes with individual volume accordingly as and when they are ready. We expect now to deal with this problem in some greater detail as we uncovered an obvious need for better information in the area and consequently the need for separate editors for each volume with me. First Volume Fruit and Vegetable Diseases has been edited by me and consists of thirteen chapters under three sections. Sections I and II deal with diseases of some important crops and their management. Major types of pathogens including bacteria, fungi, viruses and insects *etc*. causing diseases and loss have been included. Losses due to nutrient deficiency and their management has been dealt with great authority. Decrease in yield of fruit loss due to vertebrate activity and their management has also been included.

The third section is devoted to general themes giving integrated ideas and information essential for clear understanding. These chapters deal with some important mechanisms/approaches used for management of diseases.

I thank Miss Claire van Heukelom and Dr. Ir. J.A. Flipsen at Kluwer Academic Publishers, Netherlands Plant Science Unit for their encouragement, active support, cooperation and dedicated assistance in editorial structuring. I am specially thankful to Amber A. Tanghe-Neely of Kluwer Academic Publishers for final copy editing. I am looking forward to working together towards future volumes and enhancing the literature on various topics related to Management of Fruit and Vegetable Diseases.

We are indebted to all authors for their up-to-date discussions on various topics. The articles are original and some aspects have been included for the first time in any book on plant pathology. Since these chapters have been written by independent authors, there is possibility of a slight overlap/repition of certain facts but this is unavoidable in task like this.

We offer these volumes to the scientific community interested in plant diseases with the hope that these will be of great help to users.

Delhi, September 2003   K.G. Mukerji

VOLUME 1 : FRUIT AND VEGETABLE DISEASES

CONTENT

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Fruit Diseases

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Nutrient Deficiency Disorders in Fruit Trees and their Management

C. Chatterjee and B.K. Dube

ABSTRACT : The growth, development and productivity of fruit trees depend on several environmental and biotic factors. Among these factors nutrient imbalance also causes serious disorders in plants and as a result not only the yield but also quality of fruits is affected. To identify the disorders created by low or excess quantities of essential macro- and micro-nutrients, several techniques and methods have been specified which are helpful in recognizing and diagnosing the nutrient disorder(s) not only in fruit trees but also of the soil on which these are growing. Various particular parts of the fruit trees have been identified which are suggestive of nutrient status of the trees. On the basis of these, the deficient, sufficient and excess values have been worked out and are helpful in recouping such disorders. In hidden hunger conditions in addition to quantitative nutrient analysis, certain biochemical parameters also play an important role in specifying the disorder(s) specially in deficiency conditions. In abnormal nutrient conditions with the help of techniques, recovery in productivity has been obtained in several instances.

To obtain sustenance in food production, the application of both inorganic fertilizers along with organic manures is essential.

1. Introduction

In last few years nutrition of fruit trees has undergone tremendous change. Hence, new directions should be given to design fertilizer programme for these plants after considering fully the physiology of fruit trees. The mineral requirement of fruit trees can only be maintained by continuously replacing the nutrients, which is being removed by the trees. With the increase in population, the consumption of fruits is also on increase but the nutrition of trees are declining because of the use of less agricultural lands for cultivation of fruit trees and also because of the deficiencies of essential nutrients that are apparent

both on plants as well as on soils as a consequence of adaptation of modern agricultural technology and indiscriminate use of inorganic fertilizers. But at the same time implementation of several methods (agricultural practices) have benefited the ruminants as well as human beings in many ways. The use of inorganic fertilizers (synthetic) along with organic fertilizers in many forms such as synthetic chelates, natural organic complexes, organic manures and rural and urban wastes are also in vogue.

The plants are endured with the capacity for synthesizing all the biogenic molecules that makeup their structure and make them functional. Except C, H, O, all other sixteen essential elements are absorbed in molecular or ionic form *e.g.* N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, Mo, B, Co, Na, Cl and Ni by the plants. The essentiality of these elements have been defined on the basis of the three criteria laid down by Arnon and Stout (1939) and later modified by several workers.

The research on fruit tree nutrition is changing from investigation of responses to fertilizers and other supplementary nutrient applications to studies of other factors influencing nutrition. These factors are soil management, orchard design and uptake, transport and remobilization of mineral nutrients and their role in tree growth and production.

To understand the nutrient need as well as to rectify any disorder due to low or high amounts of essential nutrients of the trees, several methods have been developed. These trees are susceptible to nutrient deficiency with very marked effects on growth, leaf development and fruiting (Bould *et al*. 1949).

These culture experiments have been performed at different places by soil scientists and horticulturists using water and sand culture techniques and have greatly helped in identifying nutrient deficiencies and also in formulating the fertilizer programming.

The requirement of nutrients for fruit trees is not only for a certain organ of the plant but sufficient amount should be present within the tree at a specific time, such as in case of nitrogen application which should be done late, as this has some additive effects apart from certain disadvantages. Nitrogen sprays have been tried after shoot growth has stopped and the fruit is harvested (Shirm *et al*. 1973). In

contrast to this application of boron before or early blossom is beneficial for good fruit setting and early bud break.

To assure the transport of proper nutrients into the target organs, all tree functions should be coordinated. Such functions may fall into two general groups :

(i)   Efficient root function must be assured (Bar-Akiva *et al*. 1974) and

(ii)   in their functions competition within the tree must be altered in favour of the target organs.

In higher plants the maintenance of different physiological systems is dependent on proper nutrition, changes from its adequate or optimum concentration may cause disturbances in vital functions of plant metabolism. This may lead to some anomalies which should be corrected by diagnosis of the problem. The physiological role of most of these essential nutrients which are responsible for proper growth are almost known. With the systematic investigations on different aspects of plant metabolism the use of precise fertilizer doses for fruit trees would have better impact on economic yield.

2.   Management of nutrient disorders

Nowadays many nutritional disorders in crops can be prevented by appropriate crop management, particularly soil analysis and fertilizer use. The remedial treatments include application of nutrients directly to the soil or as foliar sprays to the crop. Stem injections of nutrients has also been used for tree crops. Fruit trees are equally prone to nutritional disorders.

Like any other agricultural crop, the assessment of fertilizer requirements of fruit trees is done by the following methods :

(i)   Experiments under controlled conditions and field trials.

(ii)   Visual characteristic symptoms of deficiency of essential nutrients

(iii)   Soil analysis

(iv)   Plant analysis

3.   Experiments under controlled conditions and field trials

The chemical properties of soil are in a major way responsible for plant behavior in fields. But the physical characteristics govern the

environment for root growth and therefore the total supply of nutrients and water of the crop is dependent on the capability of plant along with its rhizosphere (Mukerji 2002, Russell 1977). With the understanding of the physical and chemical properties of soil, the experiments on field trials as well as controlled experiments have been of great advantage to give proper direction to mannuring fertilization of not only fruit trees but other crops also. For example, development and diagnosis of major deficiency effects of essential nutrients have been possible through controlled cultural experiments. On the other hand, formulation of fertilizer recommendation are based on manipulation through field trials. This is advantageous as such experiments are usually conducted in the ideal environmental situations. These recommendations are dynamic and should be modified from time to time.

The experimental results based on pot culture and field trials in many ways, have helped the recommendation of fertilizer practices for different crop plants including that of fruit trees. These trials provide basic information to formulate the programmes more precisely. Several reports suggest that when these recommendations are applied, have given fruitful results.

4.   The role of Nutrients

4.1. Macronutrients

4.1.1. Nitrogen

Nitrogen is abundant in atmosphere and has been placed next to carbon, hydrogen and oxygen. This has been categorized as a mineral element for reasons of root absorption and uptake specially as nitrate nitrogen and also in some inorganic and organic forms. The nitrogen containing principal compounds are several different proteins, nucleic acids (DNA and RNA), amino acids, amides (Hewitt 1983), part of several porphyrin rings of chlorophyll, siro-heme, enzymes – nitrate and nitrite reductases, nitrogenase, cytochromes, hemoglobin, nucleotides (Marschner 1995) *etc.* Nitrogen takes part as reserves or protective compounds involved in different metabolic pathways of plants (Bollard 1956, Reinbothe and Mothes 1982). Nitrogen remains involved with other essential nutrients as a part of several enzymes as well as of primary and secondary compounds. Its deficiency not only causes disturbances in growth but also affects adversely several physiological systems.

4.1.2. Phosphorus

Next to nitrogen phosphorus is another primary nutrient responsible for healthy growth of plants. The principal function of phosphorus is either that of energy transfer by formation and hydrolysis of pyrophosphate anhydride bonds in nucleoside ribose triphosphates or in certain other type of energy rich anhydride bonds with enol groups Phosphoenol pyruvate (Whatley and Allen 1954, Arnon 1956, Ramarah *et al.* 1964). In assimilation of ammonium compounds and nitrogen storage products and protein amino acids depend on the presence of ATP as an energy source for their utilization and conversion to different biomolecules. In both the nucleic acids (DNA & RNA) phosphorus is a major element forming the building blocks (Hewitt 1983, Marschner 1995). The membranes of almost all cell and sub-cellular organelles contain phosphorus as phospholipids in organized association with the membrane proteins. Besides this phosphorus play many other important roles in plants (Mazliak 1973).

4.1.3. Potassium

Potassium plays an important role in balancing the negative charge or organic acids produced within the cell and of anions absorbed by roots from the external medium. Potassium functions as an activator for several enzymes (Evans and Sorger, 1966). According to Hewitt (1983) the multiple functions of potassium in enzyme systems can also explain why deficiency effects are varied in different plant species or depressed greatly on intensity of deficiency, age of plants, and availability of other elements. Another important role of potassium is in control of stomatal apereture by the movement of guard cells (Fujino 1967, Fisher and Hsiae 1968, Humble and Rasckhe 1971).

　　　The deficiency of potassium results in low protein synthesis and higher rate of respiration (Gregory and Sen 1937). The ion transport through the membranes is marked by potassium which also controls the activity of various ATPases (Fisher and Hodges 1969).

4.1.4. Calcium

The role of Ca is its involvement in the structure, stability or formation of membranes and also in behavior of nuclei and chromatin.

　　　In root cells, abnormal mitosis (Sorokin and Sommeer 1929, 1940) under Ca deficiency has been observed, chromosomes fail to separate completely and haploid nuclei occurs. Organelles or certain cell region with limiting membranes are defective or morphologically abnormal in calcium deficient plant cells (Marinos 1962, Hewitt 1963). Calcium forms salts with pectic acids at the level of cell wall composition (Hewitt 1983). It activates certain enzymes such as phospholipases (Davidson and Long 1958) , ATPases (Kalckar 1944) and enolases (Paulsen and Harper 1968). In certain enzyme activities Ca can also be replaced by either magnesium or potassium. Calcium is also known to activate nitrite permease in a membrane around chloroplast (Paulsen and Harper 1968). Calcium specially transport sequence in photosynthesis between photosystem II for O_2 evolution

and photosystem I for ferridoxin reduction (Barr *et al* 1980) Several proteins bind Ca out of which the most important being Calmodulin of general importance.

Calcium in many ways is involved in nucleotide and membrane metabolism depending on its association in several structural and chemical functions.

4.1.5. Magnesium

Magnesium is mostly involved as a constituent of chlorophylls a and b in plants. The requirement of Mg is well established in several enzyme activities especially those which are involved in photosynthesis *e.g.* ribulose –biphosphate, carboxylase, fructose biphosphate *etc.* Magnesium deficiency results in early extensive destruction of choloroplast structure (Thompson and Weier 1962, Marinos 1963, Vesk 1966, Whatly 1971) and several enzymes residing in mitochondria also degenerate in deficient conditions. Magnesium is important in the stability of ribosomal particles. The concentration is critical for both association and dissociation of ribosomal units (Ts'o 1962, Ts'o *et al.* 1950, Webster 1961). A large concentration of magnesium is involved in the structure of ribosome and in healthy plants its function corresponding to about 75% of the mineral leaf Mg concentration.

4.1.6. Sulphur

Sulphur is an integral part of two essential amino acids – methionine and cystine. In addition sulphur is a component of these compounds also which participate in cell redox reactions and also a compound in transfer of amino acids across cell membranes by a trans peptidases reaction (Hanes *et al.* 1952). Sulphur is also involved in these compounds which include flavour and volatile compounds (Synge and Wood 1956, Morris and Thompson 1956). The assimilation of sulphur to the level of thiol amino acid probably involves four and five enzymes but the opinions about these metabolic pathways are controversial (Wilson and Renveny 1976). Sulphur at certain levels of oxidation is a constituent of protein containing non– haem iron with equivalent acid labile sulphur eg. ferredoxin.

4.2. Micronutrients

4.2.1. Manganese

In plants, Mn (II) is the most dominant form and can readily be oxidised to Mn (III) and Mn (IV), therefore, Mn has an important role in redox processes (Hughes and Williams, 1988). Manganese activates several enzymes. It complexes with a special protein involved in photosystem II and also in superoxide dismutase (SOD). SOD exists in all aerobic organisms and play an essential role in the survival of these organisms (Elstner 1982, Fridovich 1983). The enzyme SOD protects the tissues from deleterious effects of oxygen free radicals O½ formed in various enzyme reactions in which a single electron is transmitted to O_2. In photosynthesizing cells the role of manganese is very sensitive as PS II system is impaired in its deficiency. Manganese is also known to activate 35 enzymes where it acts as a

cofactor (Burnell 1988). Out of these enzymes several catalyze oxidation, reduction, decarboxylation and hydrolytic reactions. In tricarboxylic acid cycle Mn has a primary role. Apart from this, Mn also activate certain enzymes of Shikmic acid pathway. Usually proteins are accumulated and carbohdrate contents are reduced in low Mn conditions. The involvement of Mn in lipid metabolism is more complicated. It is specially directly needed for proper growth of roots as in its non-availability there is shortage of carbohydrates as well as low manganese affects cell division. Manganese deficiency affects not only the economic yield but its quality also In several biochemical reactions Mn^{++} is replaced by Mg^{++}.

4.2.2. Copper

Copper is a constitutional part of plastocyanin a protein of flavin type required for light induced production of $NADPH_2$. In the deficiency of copper the rate of photosynthesis is reduced as both photosystem I and II are disturbed. The chlorophyll concentration is also affected but the ratio of chlorophyll a and b is usually increased. The chloroplastic membranes are also disturbed (Baran *et al*. 1990). Copper rudiates the lipid peroxidation processes in the photosynthetic membranes of chloroplasts (Sandman and Boger 1980). Low copper disturbs carbohydrates metabolism of plants and as a consequence of which sugars accumulate and ultimately nitrogen metabolism is also affected. Copper have some beneficial effects on fixation of molecular N_2 (Gribanov 1954) and in such plants the synthesis of proteins is also disturbed as several amino acids are accumulated. In plants Cu plays an important role in the synthesis of DNA and RNA (Ozolinya and Lapinya 1965). In plants several enzymes are either activated or have copper as an integral part *e.g.* polyphenol oxidase (Malkin and Malmstrom 1970), ascorbic acid oxidase (Powers *et al*. 1944), cytochrome C oxidase and galactose oxidase *etc.* In ribulose biphosphate carboxylase (Wishnick *et al*. 1969), copper is tightly bound to the protein component. A shortage in Cu causes a decrease in the activity of all above mentioned enzymes. Deviation in ascorbic acid oxidase enzyme in Cu stress is regarded as one of the physiological indicators of Cu status (Delhaize *et al*. 1982). The activity of one of the forms of an important enzyme *i.e.* superoxide dismutase (SOD) contains Cu in addition to Zn, is impaired when Cu stress conditions occur (Kanematsu and Asadaa 1989). Usually the activity of these enzymes (SOD) is highest in leaves of young plants. The role of copper in water stress has also been discussed by several workers (Ninh 1971, Pudova 1970, Gaina and Silli 1973, Graham 1976, Downes and Turoey 1990). In severe deficiency of Cu not only grain yield was disturbed but also induces wilting of plants and increases leaf diffusive resistance and at the same time leaf water potential also increases (Graham 1976). In its deficiency the leaves curl due to decrease in osmotic pressure which resulted indirectly in an apparent decrease in photosynthesis. The deficiency of copper reduces the rates of transpiration, respiration and photosynthesis (Koula and Cingrosova 1975). In low Cu, the lignified tissues get destructed and are unable to retain water ultimately giving a wilted appearance. Copper plays an important role in the reproduction of higher plants, particularly in cereals. Deficiency of Cu retarded the development and

generation of anthers and pollens and favours sterility. As a result, the flowers mostly abort and the reproductive parts remain under developed.

4.2.3. Zinc

Zinc is mainly required for the synthesis and utilization of carbohydrates in plants (Charters and Rolison 1951). In a large number of enzymes zinc is an integral component and have three main functions NO_2, catalysed and co-catalytic (coactive) or structural (Vallee and Auld 1990, Vallee and Falchuk 1993). In carbonic anhydrase and carboxypeptidase, zinc has a catalytic function. In alcohol dehydrogenase, zinc as structural atoms is involved with protein of the enzyme. In superoxide dismutase enzymes zinc along with copper are involved as structural component because in zinc deficient plants, the activity of the enzyme is lowered more significantly (Cakmak and Marschner 1988c).

In higher plants, several dehydrogenases, aldolases, isomerases and transphosphorylases, the role of zinc in DNA and RNA metabolism and protein synthesis has also been documented. Recently a new class of zinc dependent proteins (zinc metalloproteins are identified for DNA replication, transpiration and indirectly thus regulates gene expression. In zinc deficient plants the rate of protein synthesis and the content of proteins are drastically reduced. But at the same time, amino acids accumulate. Zinc is a structural component of ribosomes and are essential for their structural integrity. In the absence of zinc ribosomes disintegrate zinc in high amounts is required at specific sites of protein synthesis in pollen tubes. Aldolase enzyme in plants regulates the transfer of C_3 photosynthesis from the chloroplasts into the cytoplasm and within the cytoplasm the flow of metabolites *via* the glycolytic pathway (Marschner 1986). Zinc deficiency often creates disturbances in the metabolism of auxins, particularly of IAA (Indole acetic acid), but the mode of action is obscure (Cakmak *et al*. 1989). Zinc concentration of any plant has been suggested to control the tryptophan synthesis also (Salami and Kenefick 1970). In higher plants the requirement of zinc is also for maintenance of integrity of biomembranes.

4.2.4. Molybdenum

Molybdenum is known to be transient element and it shows several chemical similarities particularly with vanadium. Molybdenum is a metal component of two major enzymes in plants *i.e.* nitrogenase and nitrate reductase (M and K, 1987). Both the enzymes are complex and contains many additive components including iron and sulphur. Nitrogenase is active in N_2 fixation by several free living and symbiotic nitrogen fixing bacteria, members of cyanophyceae and some photosynthetic bacteria (Hewitt, 1963). Molybdenum deficiency in several plants leads to the accumulation of nitrate when grown in culture (Hewitt and Jones 1947, Mulder 948, Agarwala and Hewitt 1955) or in field conditions also (Wilson 1948, Wilson and Waring 1948). The activity of alanine aminotransferase is also reduced in Mo deficient plants which might suggest that Mo is individually involved in protein synthesis. Several iron containing enzymes such as catalase, peroxidase, succinic dehydrogenase, aconitase etc. are depressed in the deficiency

of Mo. On the other hand the activity of acid phosphatase is stimulated in Mo deficiency lower and higher plants. The concentration of both nucleic acids are reduced in Mo deficient plants. This might be one of the reasons for low protein synthesis. As in the case of other micronutrients, reproductive physiology of crop plants is retarded in low Mo conditions also. Deficiency of Mo creates male sterility and the seed development is also impaired by low Mo.

4.2.5. Boron

The essentiality of boron for higher plants was established long back (Aghulon 1910, Maze 1915) and since then it was also realized that boron is relatively immobile in plants. In boron deficient plants formation of lignified cell walls of potato tubers develop a brown colour (Combrink and Hammes 1972), some of the cells show abnormal cell division or some are damaged and get swollen and have enlarged brown flecks. The floral parts usually show higher boron concentration then other vegetative parts including that of leaves (Lotti *et al*. 1989).

Some evidences also show that boron regulates water relation in plants. A decrease in water potential, stomatal pore opening and transpiration rate and an increase in tissue hydration and lowering of water saturation are common features in low boron condition. Deficiency of boron results in a higher rate of O_2 uptake in leaves. With concomitant decrease in respiratory phosphorylation in boron deficient plants. Boron plays an important role in carbohydrate metabolism as starch, total and reducing sugars accumulate in low boron. A direct relationship has been noticed in the supply of boron and storage root of certain root crops (Bonilla *et al*. 1980, Agarwala *et al*. 1991). According to the hypothesis of Gauch and Dugger (1954) boron plays a key role in sugar translocation. In boron deficient plants, cessation of growth and breakdown of meristematic activity in boron deficient plants has been accounted for, by the deficiency of carbohydrates in the meristematic tissues. Boron has been suggested to play a role in translocation of growth regulators which facilitate sugar transport and not in transport of photosynthates. In low boron conditions accumulation of oxidized phenolic compounds occur in several plant species (Perkins and Aronoff, 1956). Several reports suggest a decrease in protein nitrogen and total protein, an increase in non-protein nitrogen occur in low boron plants. A negative relationship exists between boron and non-essential amino acids except histidine and tyrosine (Iqtidar and Rehman 1984). Boron in many ways influences the nucleic acid metabolism of plants. RNA content is usually decreased and no appreciable change is observed in boron deficiency. Auxins get accumulated in low boron conditions as boron plays a significant role in auxin metabolism. The internal browning in boron deficient plants has been attributed to high levels of auxin (Scienza *et al*. 1981). Boron has no direct involvement in any enzyme structure. But in deficiency of boron several enzyme activities are distributed in leaves of crop plants. The activity of peroxidase, amylase, invertase and nitrate reductase are decreased in leaves of boron deficient plants (Dutta and Mallrath 1964, Buzover 1951, Carpena *et al*. 1978, Bonilla *et al*. 1980, Ramon *et al*. 1989). The behaviour of acid phosphatase and phenylaline ammonia lyase in deficient plant species is variable.

In certain plant species the activity of α-amylase and polyphenol oxidase increase to a variable extent. The deficiency of boron is known to produce reduced number of flowers and reduced bloom diameter and affects development of male reproductive parts. Presence of adequate boron is beneficial for generation and growth of pollen tube and pollen viability.

4.2.6. Iron

Non-availability of iron in rooting medium results in the appearance of characteristic visible effects of the deficiency of the element as chlorosis of young emerging leaves (Hewitt 1963, 1983). In young leaves of iron deficiency plants, the plastids broke down, their contents get mixed up and starch grains are broken down, their contents get lysed (Jacobson and Oertli 1956). Iron plays an important role in chloroplast development and maintenance of its integrity (Jacobson and Oertli 1956, Spiller 1980, Platt-Aloia *et al*. 1983, Bennette *et al*. 1984, Terry and Abadia 1986). Iron seems to be not directly related in the synthesis of chlorophyll but it is required indirectly as the formation of the precursor ä amino-levulinic acid (Jacobson and Oertli 1956, Nason and McElroy 1963, Marschner 1995). Iron has been suggested to play a possible role in the synthesis of some specific RNA that regulated the chlorophyll synthesis (Noort and Wallace 1966). In iron deficiency degradation of chlorophyll is increased and ratio of chlorophyll a : b and increased activity of chlorophyllase in leaves of plants have been reported (Alemela *et al*. 1983). When iron is deficient, the number of chloroplast is not disturbed but the development of chloroplast is retarded and abnormal grana development and reduced number of stroma are found (Spiller 1980, Ji *et al*. 1984). The iron deficient leaves showed a decrease in chloroplast and cytoplasmic tRNAs and specific chloroplast and nucleus encoded mRNA. The role of iron in carbohydrate metabolism is still not established. But reports are available on decreased and a slight increase in reducing sugars. Nitrogen metabolism of different plant species is affected differently by low iron supply. Iron has a specific role as a constituent metal or co-factor and is known to be involved in a number of enzymes of metabolic process in different plant species. Iron as a constituent is usually associated with porphyrin, flavin or as a simple metallic ion in several biomolecules. Decrease in the activity of catalase, peroxidase, succinic dehydrogenase and aconitase are reduced in iron deficiency as iron as a constituent part is involved. In iron deficient conditions there is greater accumulation of organic acids (Wadleigh and Brown 1952, Brown 1966). Iron remains involved in the establishment of bounds between individual DNA helices and at the same time regulates denaturation of DNA (Goldstein and Gerasimova 1963). Iron deficiency changes the nucleic acids concentration in several plant species. In some others, the content of RNA is usually not disturbed by low iron.

The role of iron as a constituent part in several heme and non-heme proteins in chloroplasts is well documented (Terry and Abadia 1986, Pushnik and Miller 1989) therefore, in iron deficiency the rate of net photosynthesis is decreased (Naik *et al.* 1985). The rate of respiration in several plant species is known to decrease in iron deficiency (Hewitt 1963). Iron is an integral part of several

biomolecules involved in the processes of respiration. The effect of iron deficiency on water relations is not known properly but in lime induced chlorotric leaves, some reports are available on decrease in water potential (Hutshinson 1970) and stomatal opening. In iron deficient plants, stomatal conductance is lowered along with the decreased concentration of chlorophyll. A positive linear correlation exists between iron content and the rate of transpiration in leaves of crop plants (Szlovak and Zoltanne 1981). Iron deficiency in several plants decreases total auxin, gibberellics and cytokin content and not that of ABA in leaves. The involvement of iron has also been suggested in overcoming male sterility of crop plants.

4.2.7. Nickel

Recently nickel has been inducted in the list of essential micronutrients and has been suggested to be chemically related to iron and cobalt. Many forms of nickel are present within plants. Several reports indicate the involvement of zinc in the stimulation of generation and growth of various crop species by low concentration of nickel in the substrate. The requirement of nickel by legumes has also been established recently (Eskew *et al*. 1984).

In certain biological systems a large number of enzymes have nickel as their metal component e.g. urease and some dehydrogenases. In higher plants urease is the only enzyme which operates but nickel is not required for the synthesis of the enzyme and at the same time metal component is essential for the structure and catalytic function of the enzyme (Klucas *et al*. 1983). In nitrogen metabolism of plants, nickel has an important role. In soybean cell culture addition of nickel not only increased the growth but also increased the urease activity. No clear evidences for nickel deficiency in soil grown plants has been reported. But toxicity of nickel for survival of plants is a turning point.

5. Visual/characteristic symptoms of deficiency of essential nutrients

5.1. Macro-Nutrient Deficiencies

5.1.1. Nitrogen

From early stage the growth of fruit trees is depressed in low nitrogen and in persistent acute deficiency the plants appear spindle shaped and upright. At growing stage in persistent N deficiency, the leaves with branches and lateral branches with main stem form acute angle. The foliage are reduced in size, old leaves appear pale yellow in colour, later develop highly coloured tints of yellow, orange and red. The symptoms proceed on younger leaves. Defoliation is premature. In mature trees, there is marked reddening of bark and the fruits are hard and small. In severe deficiency die back of twigs occur and a gradual defoliation results in thin, woody appearance of tops *e.g.* in C*itrus*.

5.1.2. Phosphorus

In phosphorus deficient fruit trees the depression in growth is apparent before any visible symptoms appear. In early stages phosphorus deficient plants resemble to that of nitrogen deficiency. In low phosphorus the development of roots is most affected. The shoots are mostly devoid of branches and each branch bears few small leaves. The plant as well as leaves are erect due to stiff upright branches and petioles. The branches form an acute angle with the main stem and the small leaves with petioles. The visible symptoms of low phosphorus appear on old leaves as marginal necrosis with irregular chlorosis of interveinal areas. These affected leaves with persistent deficiency later develop bluish, purple or deep orange tint on chlorotic areas, some premature shedding of leaves also occur (Fig.1). Flowering is greatly reduced leading to poor yield.

In certain fruit trees *e.g.* in *Citrus* species some leaves show burned areas and many had a dull green, bronzed, lusterless appearance. Phosphorus deficiency in banana results in complete ceasation of elongation (in height) with rosette of leaves. The old leaves are increasingly irregular and necrotic. In addition, the affected old leaves developed different tints of colour (Twyford, 1967).

Fig. 1:   Phosphorus deficiency in Papaya. Growth highly depressed. Leaves are deshaped, reduced, wavy lamina, interveinal chlorosis on young leaves, more near the margins, lobes turn downward.

5.1.3. Potassium

When potassium is very low, the visible symptoms on old leaves appear before any growth depression. The effects appear as interveinal yellowing initiating from apex and upper margins. With increase in age, and in severe deficiency the yellowing covers entire leaf lamina and necrosis develops throughout the margins.

In due course the entire affected leaf turns necrotic and shed prematurely. In some cases minute reddish brown or brownish gray spots develop on chlorotic areas of affected leaves. These spots enlarge in size, coalesce and bigger necrotic patches are formed. Ultimately the entire leaf turns necrotic and wither. In bigger mature trees, defoliation due to potassium deficiency is being confused with senescence but potassium deficiency is also known to hasten senescence. Chapman (1968) has given a vivid picture of potassium deficiency effects in fruit trees and almost listed nineteen principal effects of low potassium. These effects are known in various citrus species (Platt, 1968), mandarin seedlings (Mani and Prakash, 1964), peach (Cullian and Waugh 1939), apple (Hewitt 1983), *etc.*

5.1.4. Calcium
Calcium deficiency in any plant is rare under field conditions. Hence the known effects are those which have been mostly produced under controlled conditions. In citrus the symptoms have been described by a large number of workers (Garner *et al* 1930, Mc Murtrey 1941, Burstrom 1968, Shear 1975, Simon 1978). The most conspicuous effects include a marked stunted and hard condition of the tree with small leaves. These leaves are often blunt and sometimes have incompletely developed tips (Bryan 1957). The roots are very much affected by low calcium.

In acute calcium deficiency (having < 1% Ca) the growth of trees is restricted, some branches die back, reduced foliation and rounded appearance of tops. Rotting of roots and vein yellowing of young leaves are more common symptoms. In severe calcium deficiency, the affected young leaves are shed prematurely followed by death of growing tips is followed (Chapman *et al*. 1965, Reed and Hass 1923).

5.1.5. Magnesium
The effects of magnesium deficiency in fruit trees are visible late. The element is mobile in plants and the old leaves are affected. The symptoms are pronounced when the deficiency is severe. In mature fruit trees, the deficiency initiates as yellow green blotch near the base of the leaf from midrib and outer edges. This yellow patch enlarges and covers almost entire lamina leaving apex and base. In persistent acute deficiency the lamina of leaves may turn completely yellow. In apple, there is great variability in the development of magnesium deficiency symptoms (Shorrocks 1964). Only a few branches are affected or the leaves from whole tree are uniformly scorched and defoliated. In banana, persistent magnesium deficiency results in blue mottling of the petioles - these are specific symptoms and are known as 'Blue'. In acutely deficient *Litchi* the leaflets are small and tiny necrotic areas appear parallel to midvain of each leaflet. The necrotic areas enlarge and some of them become continuous and consequently the entire leaflet is affected. The affected trees fail to bloom and suffer from heavy defoliation.

5.1.6. Sulphur
Sulphur being less mobile in plants, the deficiency symptoms appear on new growth. In severe S deficiency and when plants are young, the symptoms sometimes

resemble to those of nitrogen deficiency. The common symptoms of sulphur deficiency is reduction in growth, chlorotic leaves, with shorter, thinner and woody stem. Leaf size is greatly reduced and the fruiting is also affected (Jordan and Ensmiger, 1958). In some cases, the lamina adjacent to midrib appear more yellow than the rest of leaf. The affected leaves become leathery, thick and light green in colour. The new growth that emerges are already affected by sulphur deficiency as they show the symptoms.

5.2. Micro Nutrient Deficiencies

5.2.1. Iron

The deficiency of iron is usually visible on most plants at an early developing growth stage; mostly the requirement is high at that time. The most characteristic symptoms are interveinal chlorosis of young and emerging leaves initiating from apex. In fully expanded leaves, the symptoms are absent even in severely iron deficient plants. In mild deficiency the veins appear darker green, and the lamina show interveinal light yellow patches and eventually the entire leaf may turn highly chlorotic (ivory bleaching). In due course with persistent iron deficiency, the tree appears partially defoliated causing dieback of upper portion of the canopy (Figs. 2,3). If the iron deficiency occurs at young stage of the plant growth, almost the entire plant is defoliated in severe iron deficiency.

Fig. 2:   Iron deficiency in Papaya. Young leaves completely bleached. Next lower whorl of leaves showing interveinal chlorosis. Burnt apices of lobes of middle leaves.

Fig. 3: Iron deficiency in Papaya. Young leaves highly chlorotic and bleached. Interveinal chlorosis spreading to middle leaves. The tips of lobes are burnt.

5.2.2. Manganese

The most common symptoms of manganese deficiency is interveinal chlorosis of middle and old leaves. Sometimes the whole tree shows chlorosis and the mature affected leaves often give the plant a short bushy habit with golden yellow leaves. Sometimes on these plants different coloured tints appear in persistent manganese deficiency. These are common in deciduous fruit trees like mango (Fig. 4). In

Fig. 4: Acute manganese deficiency in Mango (late stage). The main and lateral branches thin, dry are devoid of leaves from apex.

Fig. 5 : Manganese deficiency in Papaya. Fading of green colour of young and interveined chlorosis of middle leaves

mild deficiency, the mature young leaves are affected which show interveinal chlorosis more on both sides of midrib (Fig. 5). With severe deficiency, some grey spots irregularly appear on the lamina towards margins and afterward near the midrib (*e.g.* grape fruits).

In banana, the young leaves develop marginal interveinal chlorosis leading to coalescent necrotic spots and finally the leaf margins show longitudinal necrotic streaks along the margins.

5.2.3. Copper

The copper deficiency symptoms in citrus are known by several names from as early as 1875. These names are referred to as die back, exantheme or ammoniation.

The copper deficiency symptoms are common in different *Citrus* species and are, distinct by development of chlorotic patches leaving green pockets at the nodes of the twigs and brownish excrescences on fruits, twigs and leaves. These are irregular in shape and mostly present on the fruit surface. Sometimes cracking of fruits occur. In severe deficiency the twig die back due to complete defoliation of young leaves at the top and the twig also appear woody. Several new growths arise from the same point giving a bushy appearance. Apart from this, chlorosis of terminal leaves and misshapen rough or brown spots appear irregularly on the chlorotic lamina of the affected middle leaves. These spots spread more towards base of the leaf. Simultaneously the young leaves also show a fading of the green colour from the margins which in time, covers the interveinal areas of the lamina. Later the colour of the spots become dark forming larger necrotic lesions and the leaves roll inward. The remaining portion of leaves show chlorosis and puckering

as in mango (Fig. 6). These symptoms spread to next upper and lower leaves *e.g.* in papaya (Fig.7).

In different fruit trees, the pattern of chlorosis varies. In some cases, 'marble chlorosis' develops on middle leaves, where interveinal chorosis and dark green areas are intermixed, the growing is more adjacent to midrib and lateral veins. The chlorotic patches later intensify and appear almost bleached white. In some other fruit trees the mature young leaves and middle leaves show network like chlorosis, the veins remain persistently green. In the third type, the symptoms are known as speckled chlorosis. Here, white yellow speckling begins near fruits with brown necrotic spots on the surface are found.

Fig.6 : Mild Copper deficiency in Mango. Lamina of young leaves highly puckered, many turn downward, irregular interveinal areas.

Fig. 7 : Copper deficiency in papaya. Diffused chlorosis of the lamina and brown
spots concentrated at margins of young leaves

In certain instances the new leaves are narrow, small, elongated with wavy margins. In severe deficiency wilting of top twigs and defoliation of young leaves occur *e.g.* in peach (Sheur and Faust, 1980). The stunting of plant growth is also apparent from early stages. In some cases in severe deficiency of Cu the young leaves form spoon or cup like structure. In papaya, a marked depression in Cu-deficient plants have been observed. The size of young and middle leaves are reduced. The leaves show diffused chlorosis and apical half of the lobes develop minute brown spots in the interveinal areas. In banana the young leaves turn yellow and they droop (Simmonds 1959).

In mango, apart from growth depression mottling of young leaves more towards apical margins are observed. Both young and middle leaves are smaller in size and appear wilted and dry due to loss of turgidity, lamina show premature drying. In some cases margins curl inward, internodes are short and thin (Figs. 8, 9).

In Cu-deficient guava growth depression is accompanied by reduction in size and chlorosis of young leaves. The margins are necrotic. The interveinal chlorosis intensifies with increase in age, as a result ultimately the leaves are entirely affected and fall off premature.

In jackfruit also, Cu-deficiency symptom are similar to that of guava and mango, and almost follow the same pattern. In severity the upper branches show dieback type symptoms. These symptoms appear on those plants which were grown in refined sand to find out the critical limits and deficiency values of these plants in low copper (Agarwala *et al.* 1991).

Fig. 8 : Copper deficiency in Mango. Fading of colour from middle leaves. Entire apical portion of main lateral branches are dry and bear no leaves.

Fig. 9 : Copper deficiency in mango. Young leaves showing inward curling, drying up from the tip followed by necrosis forming a hook. Apex of each branch is devoid of leaves.

5.2.4. Zinc

Zinc deficiency in plants is a common disorder as compared to other nutrient deficiencies. Several fruit trees, such as citrus, apple, tung, mango *etc* are susceptible to low zinc. Zinc deficiency disorders in plants have been given specific names such as, 'mottle leaf' 'little leaf' 'frenching' and 'follocellosis'.

In *Citrus*, the symptoms appear mostly on young and middle leaves as irregular yellowing between the veins. Later the affected leaves become entirely yellow with very few small patches of green colour more near the veins. The symptoms first appear on younger growth. In due course with intensification of symptoms, the affected leaves showed mottled appearance and also remain small. Sometimes there is premature falling of leaves and dieback of shoots.

In certain fruit trees *e.g.* apple the appearance of deficiency symptoms are late almost at the time of flowering. The affected shoots have sparse foliage, with short internodes, young leaves appear rosette like and die back. In pears, the size of young leaves are reduced with a tendency for edges to curl upward, no waviness in margins of affected leaves (Fig. 10).
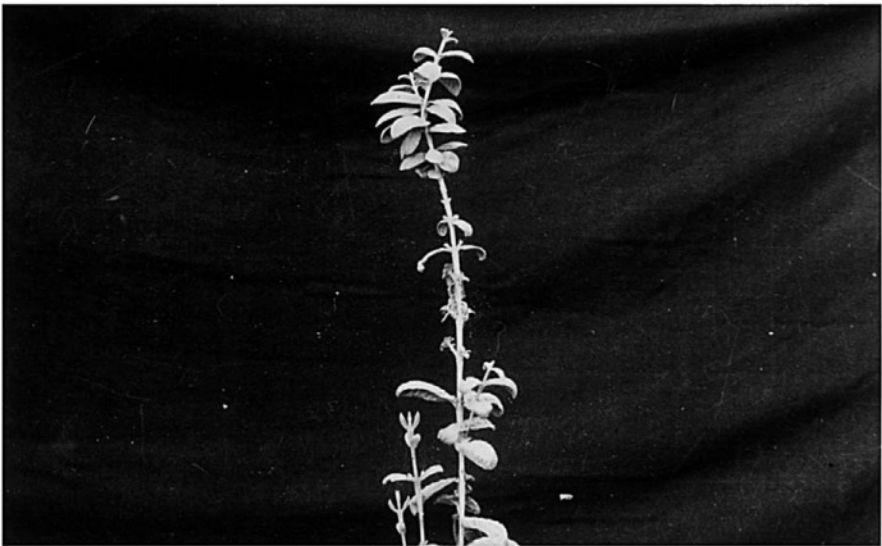

Fig.10 : Zinc deficiency in Guava. Shortening of internodes, leaves closely set, minute almost no petiole, stem thin and slender.

In mango orchards, the appearance of zinc deficiency type symptoms are more prevalent due to less concentration of zinc as most of the soils are low in zinc. The deficiency initiates on terminal growth on the upper part of the tree. The affected leaves are stiff, narrow and reduced with interveinal chlorosis. The tips and margins of the curled leaves become chlorotic. In severe deficiency, growth is completely checked and death of large twigs and even of branches also occur.

5.2.5. Molybdenum

Molybdenum deficiency usually occurs on old leaves and late almost after flowering. The old leaves show interveinal chlorosis in the form of round spots of irregular size on the lamina of leaves. Sometimes these spots appear water-soaked and in others these spots enlarge in size with more intense chlorosis changing to yellow. In badly affected leaves these spots enlarge in size and the affected leaves show mottling. These symptoms gradually spread to next upper leaves. In severity, the chlorotic spots develop a narrow round circle of necrotic tissue which completely surrounds it. The chlorosis changes to ivory bleaching and gradually the tissue from the center dissolves and a hole appears. This hole is later covered by 2-3 necrotic circular bands. In *Citrus* the roots are stunted, less developed, thick with swollen tips. In certain other cases, old leaves show diffuse mottling and develop light brown areas of dead tissue at tips and margin (*e.g.* plum). In persistent molybdenum deficiency the plants are severely restricted in growth. Molybdenum deficiency affects formation and development of fruits. Sometimes the new growth show uniform chlorosis (of young leaves).

5.2.6. Boron

The deficiency of boron greatly affects the fruit setting and reduces fruit yield drastically. This deficiency is characterized by malformed and hard misshapen fruits with black necrotic spots. The fruits may crack with roughening of the skin. Internally the fruit may develop corky areas in the cortex and browning in the cork region.

Some of the affected leaves are shed prematurely and the bark may also split. The twigs and branches show apical death and due to which they dry and wither. Boron deficiency is more pronounced on young growth and usually the apical portion is affected. In severe deficiency, further growth of plants is almost checked and due to which the internodes are short with development of numerous lateral branches (Fig.11).

Fig.11 : Boron deficiency in mango. Apical necrosis of the main stem and its branches, development of large numbers of lateral shoots, also with necrotic growing points.

In papaya, acute B deficiency depressed the growth, young and middle leaves are reduced in size, in due coarse lamina of most upper leaves, remain puckered, deshaped and small, thick, flaccid as a result the leaves appear brittle. The petioles show longitudinal cracking. In prolonged acute deficiency the plant appears short with condensed apical growth, a large number of underdeveloped lateral branches with distorted leaves appear. Simultaneously the stem turns black and the petioles of leaves arising from the apex become necrotic and black. In certain instances, in severe deficiency the stem apex collapses and axillary branches developed just below it, which with time become black and necrotic.

In guava and mango, sometimes, the terminal upper ends of branches/twigs are devoid of leaves and give a blackened look. In persistent severe deficiency, growth of plants are highly affected.

6.   Soil Analysis

Different type of soils have been found to vary in their ability to supply nutrients to plants, but these variations are not related to the total concentration of elements in soils. The availability of plant nutrients are measured by soil analysis but it can also provide a data of total concentration of element present in the soil in a long way.

The supply of plant nutrients has been suggested to be controlled by the chemical properties of soil directly. Various tests to assess the fertility status of soil with regard to macro- and micronutrients has been described as details of analytical methods have been worked out by several workers (Walkley and Black 1934, Jackson 1958, Hesse 1971).

Several factors, such as root stocks, deep root systems of fruit trees, excess and low concentration of nutrients, scion-stock combination, microbiological and climatic effects and plant needs at different growth stages, should be considered and incorporated to formulate fertilizer programme for fruit trees. At the same time, it has been observed that soil analysis in conjunction with plant analysis provides a sound basis for guidance for fertilization of an orchard – a map of sites of soil samples on which the orchards are situated should be made available for analysis and proper record of the data should be maintained.

These chemical analysis are helpful in assessing and delineating soil fertility status on available nutrients. Nowadays, incidences of

micronutrient deficiencies along with macronutrient are increasing and several suitable tests for diagnosing and assessment of such deficiencies can be employed for delineation of soil fertility. These would be helpful for monitoring and making practical recommendations for optimum nutrient requirement of soils.

For soil chemical analysis the methods are very precise and should be followed in a systematic way. The soil tests are based on Viet's approach which describes the amount of nutrient in a definite chemical form *viz* (a) Water soluble (b) Exchangeable (c) Chelated or complexed (d) Secondary clay minerals or oxides and (e) Primary minerals. The first three pools are thought to be important in supplying micronutrients to the plant during a growing season. The available micronutrients, therefore, do not reflect their total content in soils.

6.1. Macronutrients

The available N concentration can be estimated according to Subbiah and Asija (1956).

The available concentration of P is measured by the method of Olsen *et al*. (1954), by extracting with 0.5 M $NaHCO_3$ and that of Ca, Mg and K was extracted in 1 N ammonium acetate (Jackson, 1958). The available S can be extracted in 0.15% $CaCl_2$ and estimated by the method of Williams and Steinbergs (1959).

6.2. Micronutrients

As far as micronutrients such as Fe, Mn, Cu, and Zn are concerned, the widely used DTPA method of Lindsay and Norvell (1978) is adapted to extract them and estimate by Atomic Absorption Spectrophotometer to know the status of available contents. Whereas for available Mo, extraction is done by Grigg's method (1953) and for available boron an extractant suggested by Berger and Truog (1944) is used and boron is estimated by Wolf's (1971) method using Azomethene-H. These tests are helpful in generating basic information on the status of soils. Samples from different soil depths are analysed to get a better idea of the nutrient status of soil in relation to deeper root systems of fruit trees. The status of nutrients in plants (before fertilizer application) can also be assessed by chemical analysis

of plant sample, after wet digestion with di-acid mixture, (HNO_3 : $HClO_4$ – 10 : 1), (Piper, 1942).

Many soil scientists suggest that soil analysis is not very accurate and always cannot be relied upon wholly for formulating a fertility programme or diagnosing any nutrient disorder in orchards.

7.   Plant Analysis

A diagnosis based on symptoms and confirmed by chemical tissue analysis are the most reliable methods of diagnosing nutrient disorders.

Alderich (1967) has defined the important aspects of plant analysis based on the following :

- To identify or diagnose visible symptoms
- To identify hidden hunger
- To identify areas of incipient deficiencies.
- To indicate whether applied nutrients entered the plant or not.
- To indicate interactions or antagonisms among nutrients.
- To aid the understanding of internal plant functioning.
- Analysis of plant materials provides an idea of the nutrient concentration and (when multiplied by dry matter) of the total uptake.

The adaptation for leaf (tissue) analysis in perennial horticultural crops has proved its superiority over other diagnostic methods (Table 1).

TABLE 1

Common soil tests and critical levels of nutrients in soil and plants.

Element	Soil test method	Critical level in soil	Critical level in plant
Sulphur	0.15% $CaCl_2$	8-30 ppm	< 0.15-0.2%
Calcium	Amm. acetate	< 0.25% of CEC	< 0.2%
Magnesium	Amm. acetate	< 4% of CEC	< 0.10-0.2%
Zinc	DTPA	0.6 ppm	<15-20 ppm
Copper	DTPA	0.2 ppm	< 4 ppm
Iron	DTPA	4.5 ppm	< 50 ppm
Manganese	DTPA	2.0 ppm	< 20 ppm
Boron	Hot water	0.5 ppm	< 20 ppm
Molybdenum	Ammonium Oxlate	0.2 ppm	< 0.1 ppm

7.1. Sampling and Sample Preparation

For plant analysis to be more meaningful, collection of particular plant part at the right stage of growth as pre-technical specifications is very important. It would be wrong and wasteful to just pluck any leaf or branch from a growing plant at any time and send to laboratory for analysis.

Sometimes the deviation in the sampling procedure under certain specific condition becomes necessary to understand, the uptake, translocation and utilization of certain essential nutrients. The index tissue may be helpful in diagnosing of nutrient deficiency, monitoring efficient nutrient management for higher economic yield as well as for improved quality (Table 2).

TABLE 2

Plant tissue sampling for different fruit tree crops (Tandon, 1993).

Crop	Index tissue	Growth stage/ time	Sample size
Banana	Petiole of 3rd open leaf from apex	Bud differentiation 4 month after planting.	15
Citrus	3-5 month old leaf from new flush. 1st leaf of the shoot	June	30
Guava	3rd pair of recently matured leaves	Bloom stage August or December	25
Mango	Leaves + petiole	4-7 month old leaves from middle of shoot	25
Papaya	6th petiole from apex	6th month after planting	15

Leaf analysis can be misleading sometimes especially when phloem-immobile elements are dealt with, *e.g.* Ca, as deficiency and adequacy may exist simultaneously in different parts of the same plant (Loneragan *et al*., 1976). In Ca or Cu deficiency, the young leaves in deficient range of either element, is a better indicator of nutrient status, whereas old leaves usually contain sufficient Ca or Cu.

The basic principal behind this technique is that the nutrient concentration of plants is related to the amount of nutrient element available in soil (Table 3).

TABLE 3

Deficiency, sufficiency and toxic limits of nutrient for some
important fruit tree crops (Tandon, 1993)

Crop	Macronutrient	Deficient	% Tissue Sufficient	Excess
Papaya	N	0.8-1.0	1.01-2.5	> 2.5
	P	0.18-0.21	0.22-0.40	> 0.4
	K	2.80-3.2	3.3-5.5	> 5.5
	Ca	< 1.0	1-3	> 3.0
	Mg	< 0.4	0.4-1.5	> 1.2
	S	0.17-0.40	0.20-0.40	> 0.4
Mango	N	0.70-0.99	1.0-1.5	> 1.5
	P	0.05-0.07	0.08-0.25	> 0.25
	K	0.25-0.39	0.40-0.90	> 0.90
	Ca	1.0-1.99	2.0-5.0	> 5.0
	Mg	0.15-0.19	0.20-0.50	> 0.50
	S	0.05-0.19	0.20-0.60	> 0.60
Banana	N	2.0-2.49	2.5-3.0	> 3.0
	P	0.14-0.17	0.18-0.40	> 5.0
	K	2.0-2.29	2.30-4.0	> 4.0
	Ca	0.40-0.69	0.70-1.4	> 1.4
	Mg	0.40-0.69	0.70-1.4	> 1.4
	S	0.20-0.25	0.25-0.40	> 0.4
Citrus	N	1.90-2.19	2.2-2.7	> 2.7
	P	0.08-0.09	0.1-0.3	> 0.3
	K	0.70-0.99	1.0-2.0	> 2.0
	Ca	1.0-1.49	1.5-4.0	> 4.0
	Mg	0.15-0.19	0.2-0.5	> 0.5
	S	0.20-0.24	0.25-1.4	> 1.4
Guava	N	2.1-2.59	2.6-3.0	> 3.0
	P	0.11-0.15	0.16-0.22	> 0.23
	K	1.20-1.59	1.6-1.22	> 2.2
	Ca	1.0-1.49	1.50-2.60	> 2.6
	Mg	0.20-0.29	0.30-0.75	> 0.75
	S	< 0.2-0.24	0.25-0.40	> 0.4

Leaf samples for analysis should be selected on the basis of physiological age *i.e.* developmental stage. It is also important that the samples must be free from diseases, insect damage and physical or chemical injury. Leaf near the fruit should not be sampled as the nutrients that might have contained by the leaf are often translocated to the fruits

Nicholas (1957) in one of his experiments observed that chemical test for leaf analysis is the only certain way of differentiating between pathogenic and non-pathogenic (nutrient disorder) diseases.

Different plant parts such as, leaves, shoots and even entire plants (annual crop) can be used for chemical analysis. In case of fruit trees, leaf tissue is the best tissue for sampling as any abnormality (either deficiency or toxicity) is best reflected by the changes in the leaf physiology. Procedures for taking leaf samples of a definite age and aspects of a tree have been standardized for meaningful comparisons and interpretations (Table 4).

TABLE 4

Deficiency, sufficiency and toxic limits of nutrient for some important fruit tree crops (Tandon, 1993).

Crop	Macronutrient	Deficient	ppm Tissue Sufficient	Excess
Papaya	Fe	20-24	25-100	> 100
	Mn	10-19	20-150	> 150
	Zn	10-14	14-40	> 40
	Cu	< 4	4-10	> 10
	B	< 20	20-30	> 30
	Mo	0.15-0.19	0.20-20	> 20
Mango	Fe	25-49	50-250	> 250
	Mn	25-49	50-250	> 250
	Zn	15-19	20-200	> 200
	Cu	5-6	7-15	> 50
	B	20-49	50-100	> 100
	Mo	0.01-0.04	0.05-1.0	> 1
Banana	Fe	80-99	100-300	> 300
	Mn	150-199	200-2000	> 2000
	Zn	10-12	13-50	> 50
	Cu	4-5	6-30	> 30
	B	25-49	30-100	> 100
	Mo	0.03-0.29	0.3-3.0	> 3
Citrus	Fe	50-59	60-100	> 100
	Mn	15-19	20-200	> 200
	Zn	15-19	20-50	> 50
	Cu	3-4	5-100	> 100
	B	22-24	25-60	> 60
	Mo	0.2-0.4	0.5-0.8	> 0.8
Guava	Fe	50-59	60-250	> 250
	Mn	20-29	30-100	> 100
	Zn	20-24	25-200	> 200
	Cu	3-4	5-20	> 20
	B	17-19	20-70	> 70
	Mo	—	—	—

7.2. Bark Analysis

In fruit trees, in absence of leaves, bark analysis can be helpful for assessing nutrient status, *e.g.* in apple and pear, where dieback of shoots is caused by boron deficiency (Bould *et al*. 1953, Rogers *et al*. 1965), this technique is helpful for phosphorus status of certain fruit trees or accumulation of manganese in localized areas in the bark of apple shoots (even before appearance of interveinal bark necrosis) give better indication of tissue concentration (Table 5).

TABLE 5
Bark nutrient concentrations associated with tree disorders
(Hewitt, 1983)

Plant	Element	Conc. µg/g d.m.	Disorder	Reference
Apple	Mn	187-476	Internal bark necrosis	Shelton & Zeiger, 1970
"	"	$\equiv$ 600	Internal bark necrosis	Berg et al., 1958
"	"	1574	Pimply bark and necrosis	Negai et al., 1965
"	"	345	Pimply bark	Bould & Bradfield, 1954
"	"	22	Normal	Bould & Bradfield, 1954
"	Cu	1.3-1.6	Shoot die-back	Bould et al., 1953b
"	"	5	Normal	Bould et al., 1953b
"	Zn	2.1->10	Little leaf and resetting	Bould et al., 1953a
"	"	20	Normal	Bould et al., 1953a
Apricot	B	63-206	Shoot die-back	Eaton et al., 1935, 1941
Plum	"	412	Shoot die-back	Eaton, 1935
Rubber plant	P	0.048-0.316% P_2O_5	Soil-P supply assessment	Bolle-Jones, 1957

7.3. Fruit Analysis

Calcium and boron deficiency disorders in some fruits are best confirmed by chemical analysis of the affected fruits. Bitter pit in apple, a calcium deficiency disorder is specified by depression in the skin

associated with sub-surface necrotic areas in the apple cortex. In certain varieties of apple the necrotic areas might be confirmed with internal cork caused by boron deficiency. Fruit or peel analysis is used to differentiate between these two maladies. A highly significant linear relationship between calcium in peel and incidences of bitter pit (at maturity) has been observed (Drake *et al*. 1966).

External and internal cork formation in boron deficient apple and severe fruit distortion and shallow depressions in fruit surface of pears, malformation and cracking in severely affected fruits in boron deficiency are usually confirmed by boron content of fruits and is reliable method from distinguishing it from that of calcium (Table 6).

TABLE 6
Nutrient concentrations related to fruit disorders (Hewitt, 1993)

Plant	Element	Tissue	Nutrient concentration Mg	Disorder	Reference
Apple	Ca	Fruit	<5.0 mg/100 g fr.wt.	Bitter pit	Van Goor, 1971
"	"	"	<5.0 mg/100 g fr.wt.	" "	Perring & Jackson, 1975
"	"	"	<2.5 mg/100 g fr.wt.	" "	Wills et al., 1976
"	"	"	<4.7 mg/100 g fr.wt.	" "	Das and Van der Boon, 1972
"	"	"	<3.0 mg/100 g fr.wt.	Senescent breakdown	Perring, 1968
"	"	Apple peel	<700 µg/g dry wt.	Bitter pit	Drake et al., 1966
"	"	"	<500 µg/g dry wt.	" "	Chiu and Bould, 1977
"	"	"	<700 µg/g dry wt.	Normal	Chiu and Bould, 1977
"	B	Fruit	<8 µg/g dry wt.	Internal cork	Askew, 1935
"	"	"	3-5 µg/g " "	"	Burrell et al., 1956
"	"	"	5 µg/g " "	"	Demetriades et al., 1963
"	"	"	4 µg/g " "	External cork	Demetriades et al., 1963
"	"	"	>10 µg/g " "	Normal	Chiu and Bould, 1977
Pear	"	"	12 µg/g " "	Surface cracking	Johnson et al., 1955

8.   Biochemical Parameters as Assessment Tool for Nutrient Status of Fruit Trees

The role of essential metal nutrients is important in plant metabolism as they stimulate interest in biochemical methods for diagnosing nutrient deficiencies specially under hidden hunger conditions, whereas abnormal concentrations of plant metabolic products associated with nutrient disorders may also indicate abnormal condition of the plant.

As far as metal requirement of plant enzymes are concerned, they have been grouped in two broad classes (Hewitt 1963):

(i)   Those enzymes in which a specific metal has been shown to be an integral component.

(ii)   Those enzymes for which one or more metals serve as an activator (Nason and McElroy, 1963). Some of the essential nutrients (specially micronutrients) are usually an integral part of the enzymes whereas magnesium and manganese are frequently involved as activator.

A hypothesis, widely accepted, has been proposed for the first time by Brown and Hendrick (1952) which gives a basis for assessing the nutrient status of plants depending on enzyme activity. The hypothesis indicates "If an element is limiting in the nutrition of plant, the deficiency will be evident in changed enzyme activity, as the enzyme requires that particular element for its function" *e.g.* the activity of ascorbic acid oxidase is markedly reduced by limited copper supply or catalase is reduced when iron supply is low or activity of peroxidase is increased markedly in manganese deficiency and decreased significantly when iron limits. Bar-Akiva (1961) has stated that the activity of peroxidase can be an assessing indicator of the status of manganese in plants. Kessler (1961) observed that the activity of ribonuclease can be an index of zinc availability for fruit trees. This view was supported by Dwivedi and Randawa (1974) also. The use of pyruvate kinase enzyme activity can be helpful in ensuring and diagnosing the optimal levels of potassium or magnesium even before the onset of visible deficiency symptoms (Besford 1975).

Bar-Akiva *et al*. (1976) suggest that pyruvate activity serve as a more objective and less emperical indicator of cation balance in plants. Similarly the activity of nitrate reductase has been used for predicting nitrogen requirement of several crop plants (Johnson *et al*. 1976). For assessing nutrient status of trees and crop plants, it is also suggested

(Steward and Durzan, 1965) that it is beneficial to study the chemical changes that occur within the plant on re-supplying the nutrient and then diagnose its requirement also (Table 7).

TABLE 7

Corrective measures for micronutrient deficiencies (Tandon, 1993).

		Spray (%)	Soil application
Zn	Zinc sulphate	0.1 %	25 kg/ ha
Mn	Manganese sulphate	0.5 %	20 kg/ ha
Cu	Copper sulphate	0.1 %	10 kg/ ha
Fe	Ferrous sulphate	1 %	20 kg/ ha
B	Borax	0.1 %	10 kg/ ha
Mo	Sodium molybdate	0.1 %	10 kg/ ha

9. Recommendations

Suppression or acceleration of several activities including enzyme activity may give rise to either accumulation or lowering of certain metabolic products. This is more common with nitrogen containing compounds present in low or high concentration in any part of plants and may indicate, the deficiency or excess of any essential nutrient. Some other chemical compounds also show similar trend (Samish and Hoffman 1966, Taylor and May 1967, Bar-Akiva 1971).

10. Issues And Strategies To Meet The Challenges

Research should be intensified on the issues mentioned below :

1. Preparation and updating of thematic maps of macro- and micronutrient deficiencies.
2. Enhancing micronutrient availability and use efficiency.
3. Improving fruit quality by application of micronutrients.
4. Systematic research is very much needed in monitoring of micronutrient deficiencies.
5. Information on micronutrients in soils, areas so far remain uncovered should be covered.

6.   Suitable models for forecasting emerging micronutrient deficiencies, their transformation and residual availability and soil pollution needs to be developed.

7.   Monitoring the effect of micronutrient in soil, plant, animal, and human chain needs special attention.

8.   The correction of micronutrient deficiencies and toxicities is very crucial to achieve the target to produce 200 millions of fruits by 2025 A.D. This would only be possible if the extension services provided by several agencies are better coordinated, improved and strengthened, for this purpose the following issues need to be considered and adopted.

- Providing micronutrient soil testing advisory services.
- Human resources development to provide better and efficient advisory services.
- Dissemination of information on micronutrient technology.
- Improving micronutrient production supply.
- Quality control on micronutrient fertilizers.

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2

Apple Scab and its Management

Ralph L. Nicholson and James E. Rahe

ABSTRACT : Apple scab caused by the fungus *Venturia inaequalis* (Cke.) Wint. is a destructive disease of apple. The pathogen is a facultative saprophyte that grows subcuticularly on the host. *V. inaequalis* must obtain nutrients through an active means. The fungus grows as a stroma of thick-walled cells between the cuticle and the outer wall of the host epidermis. Initial infections can lead to production of conidia on infected tissues within 9 to 17 days. The asexual spores can cause numerous secondary infections. Several waves of secondary infection can occur during a single growing season. Complete crop loss can result and severe infection can reduce blossom bud formation and crop potential for the following year, which may promote biennial bearing. Scab management is an essential component of orchard management in climates that are conducive to infection. Fungicides that are currently available for control of apple scab can be categorized as either protectant or eradicant in nature.

1.   Introduction

Apple scab caused by the fungus *Venturia inaequalis* (Cke) Wint. is one of the most destructive of apple diseases. This chapter will consider literature pertinent to biology of the pathogen and current control strategies. Considerable information is available on the genetics of the pathogen and host and the reader is directed to the following references (Bolar *et al.,* 2000, Hemmat *et al.,* 1998, Le Cam *et al.,* 2002, Xu and Korban 2000, Xu *et al.,* 2001).

The pathogen is a facultative saprophyte that grows subcuticularly on the host (Nusbaum and Keitt, 1938). Because of this growth habit the pathogen must obtain nutrients through an active rather than passive mechanism (Nicholson, 1972, Nicholson *et al.,* 1977). The fungus grows as a stroma of short, thick-walled cells between the cuticle and the outer wall of the host epidermis (Figs. 1, 2, 3).

Germination, appressorium formation and penetration are the same on all apple hosts regardless of their resistance or susceptibility to the pathogen. In susceptible interactions, the stroma becomes thick and can cover an extensive area of host tissue, (Fig. 2). In resistant interactions, growth of the fungus is limited. In hypersensitive interactions, growth may be limited to only a few cells (Fig.4) (Nicholson, 1972).

Maeda (1970) demonstrated that the appressorium of the fungus contains a unique structure that she termed the appressorial infection sac (Fig. 5). Nicholson *et al.* (1972) demonstrated that during the germination process the conidial germling exhibits a transitory appearance of non-specific esterase enzyme activity (Fig. 6). Subsequently, one of the esterase enzymes was shown to be a cutinase (Köller and Parker, 1989) and this is consistent with the subcuticular growth habit of the fungus. The fact that cutinase is produced by the fungus was indirectly demonstrated first by Maeda (1970) who showed microscopic evidence that the fungus actively degrades the cuticle (Fig. 5). Cell wall degrading enzymes seem not to be particularly significant to the pathogen although literature suggests that *V. inaequalis* like other fungal pathogens produces cell wall degrading enzymes (Kollar, 1994, 1998).

Recently, Aylor reviewed the means through which the fungus is dispersed as well as some of the current strategies for disease control (Aylor, 1998). MacHardy (1996) also published a thorough review of the disease and its management.

It has recently been shown that apple contains receptor-like genes that are like the *Cladosporium fulvum* resistance genes in tomato (Vinatzer *et al*, 2001). Three members of the cluster were sequenced completely. As with the Cf gene family of tomato, the amino acid sequences coded by these genes contained an extracellular leucine-rich repeat domain and a transmembrane domain. It is interesting that Bolar *et al.* (2000) reported that in transgenic apple endochitinase from *Trichoderma harzianum* increased the level of resistance to apple scab. In related work, Xu *et al.* (2001) used a bacterial artificial chromosome (BAC) library of *Malus floribunda* 821 to investigate the apple scab resistance gene Vf. The resistance gene Vf, from the wild species *Malus floribunda* 821, was incorporated into a variety of apple cultivars through classical breeding. The aim was to isolate

the Vf gene. A bacterial artificial chromosome (BAC) library of 31,584 clones was constructed. Analysis of randomly selected clones showed the average insert size at 125 kb. If it is assumed that the genome size of *M. floribunda* 821 is 769 Mb/haploid, the library represents about 5x haploid genome equivalents. This provides a 99% probability of finding any specific sequence from this library. PCR-based screening of the library has been carried out using eight random genomic sequence-characterized amplified regions (SCARs), chloroplast- and mitochondria-specific SCARs, and 13 high-density Vf-linked SCAR markers. An average of five positive BAC clones per random SCAR has been obtained, whereas less than 1% of BAC clones are derived from the chloroplast or mitochondrial genomes. Most BAC clones identified with Vf-linked SCAR markers are physically linked. Three BAC contigs along the Vf region have been obtained by assembling physically linked BAC clones based on their fingerprints. The overlapping relatedness of BAC clones has been further confirmed by cytogenetic mapping using fiber fluorescence *in situ* hybridization (fiber-FISH). The *M. floribunda* 821 BAC library provides a valuable genetic resource not only for map-based cloning of the Vf gene, but also for finding many other important genes for improving the cultivated apple. In related work, Xu and Koerban (2000) reported saturation mapping of the Vf gene for scab resistance.
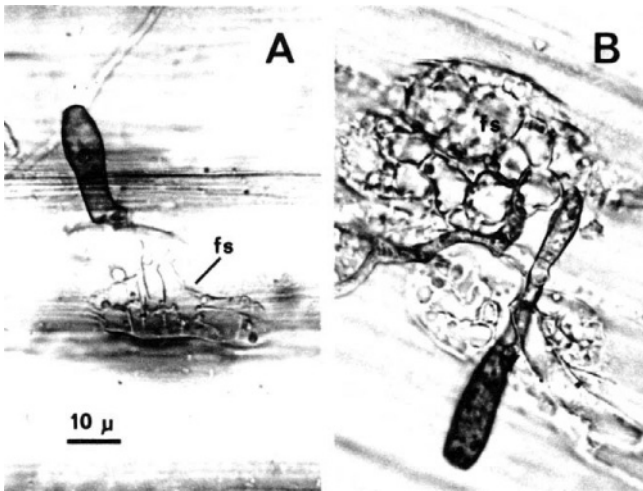

Fig. 1. A) Developing subcuticular fungal stroma (fs) on an etiolated apple hypocotyl. B) Fungal stroma several cells in thickness on an etiolated apple hypocotyl.

Fig. 2.   Subcuticular development of *V. inaequalis* on a susceptible leaf. A. Subcuticular hypha in transverse section (FC), cuticle (c), host wall (HW), Osmiophilic droplet (OD), arrow (plasmodesmata). B. One layer thick stroma, C (cuticle), FC (fungal colony), Host wall (HW), Epidermal cell (E). From Figure 9 by Maeda (1970).
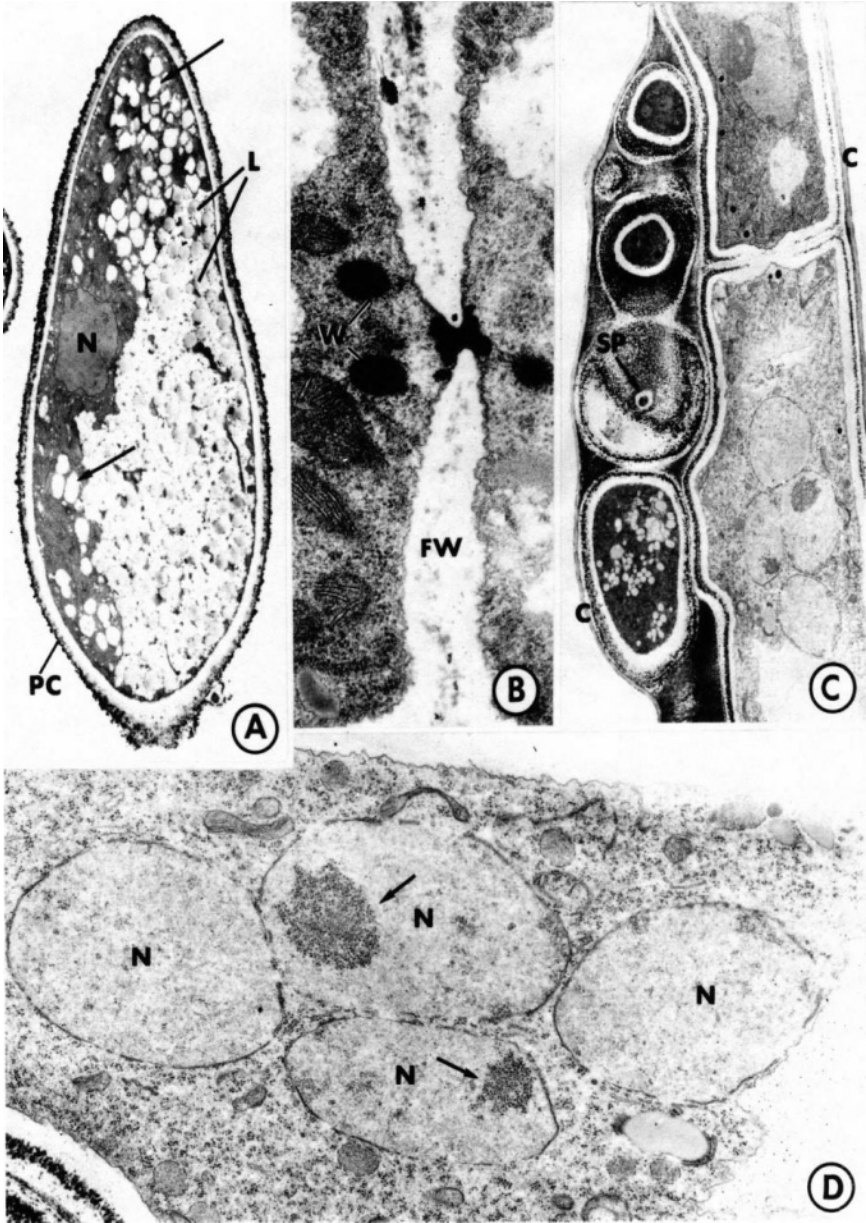
Fig. 3. A. Ungerminated spore, N=nucleus, L=lipid bodies, PC = electron dense particulate coat.   B. Longitudinal section through stroma, W =Woronin bodies, FW = septal wall layers. C. Stroma on hypocotyl cells C = host cuticle, SP = septal pore, D. Cluster of nuclei (N), arrows indicate nucleoi. From Maeda, 1970.
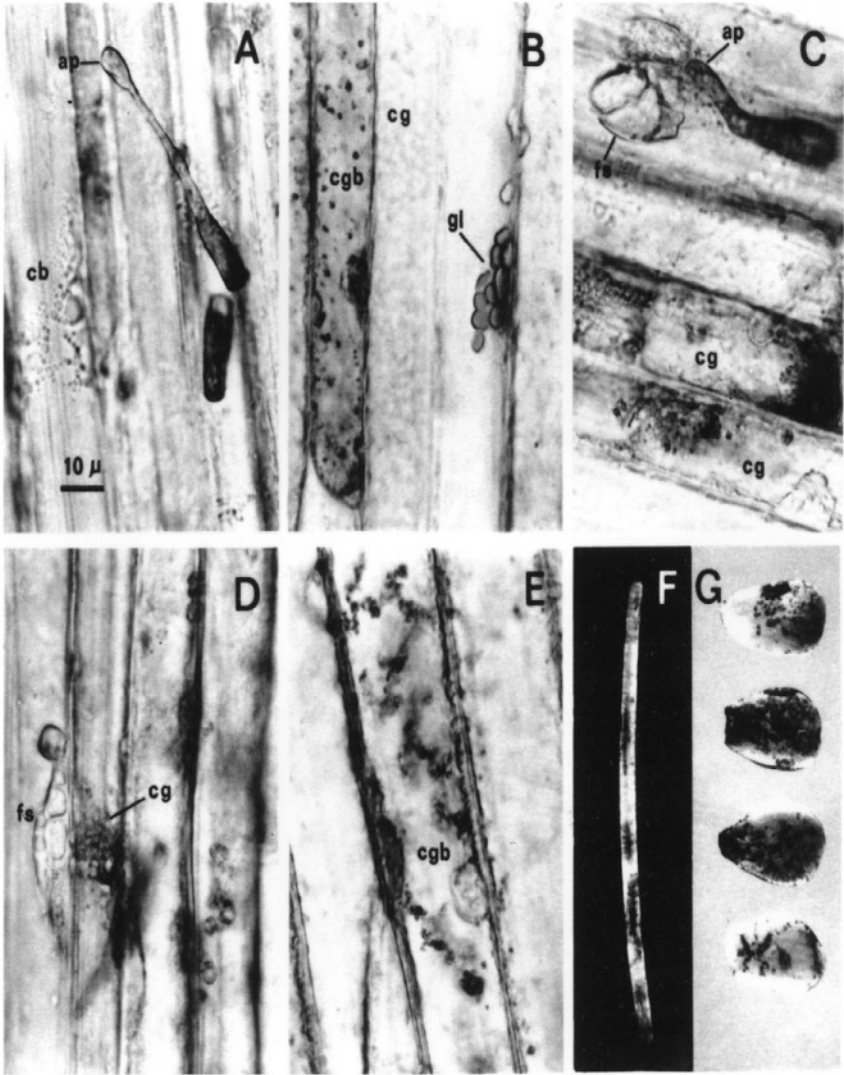
Fig. 4. The hypersensitive apple scab interaction on etiolated apple hypocotyls. A) Cytoplasmic bubbling (cb), B) host cytoplasmic granulation (cg), yellow globules (gl), extreme granulation and browning (cgb), C) Host granulation (cg) and limited stromatic growth of the fungus (fs), appressorium (ap), D) Cytoplasmic granulation (cg) and limited growth of the fungus (fs), E) Extensive granulation and browning, F) Coalesced hypersensitive lesions on etiolated hypocotyls, G) Coalesced hypersensitive lesions on cotyledons.
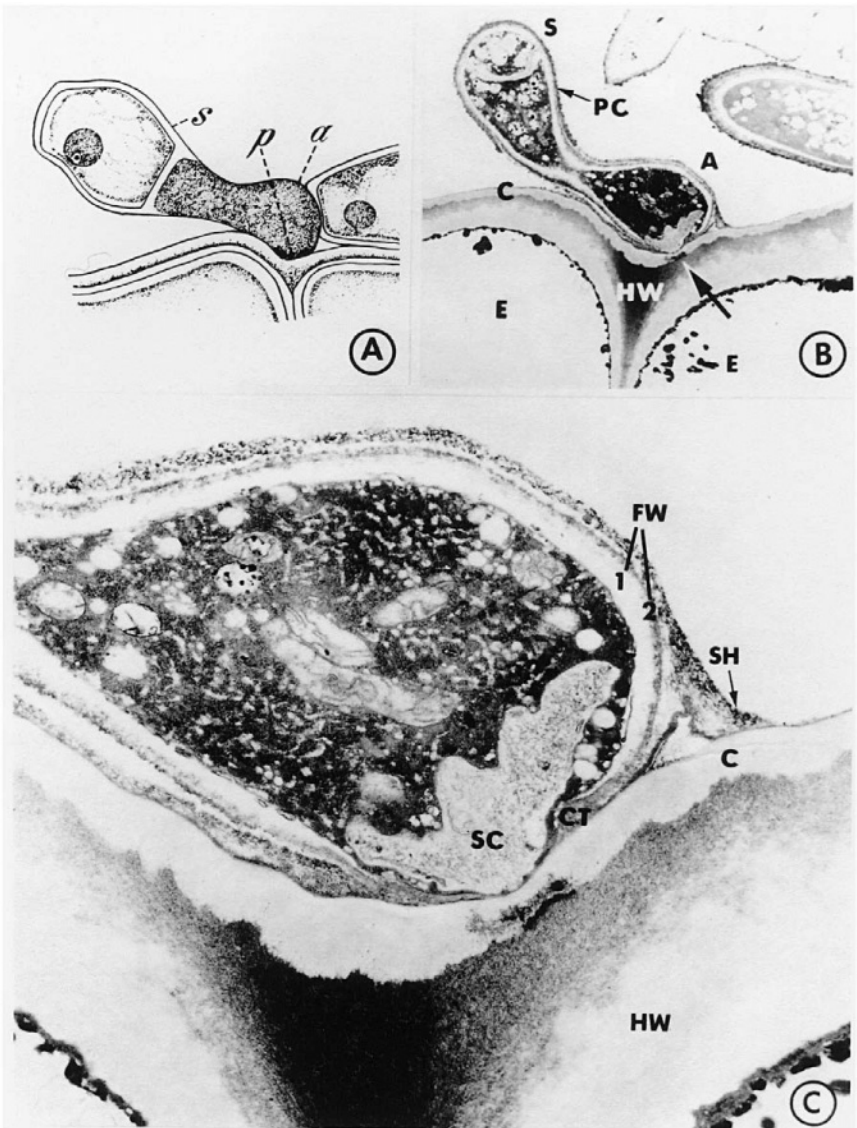
Fig. 5. Penetration site of *V. inequalis*. A. Reproduction of figure 2K from Neusbaum and Keitt (1933). spore (s), penetration site (p), appressorium (a). B. Micrograph, apple leaf fixed 20.5 hr after inoculation. Penetration along anticlinal wall junction of epidermal cells (HW). x 3000 spore (s), spore (s), pc (particulate electron dense coat), E (epidermal cell). C. Electron micrograph of appressorium, with infection sac (SC) sheath (SH), fungal wall layers 1 and 2 (FW), thickening bordering the pore (CT), cuticle (C). From Maeda, 1970.
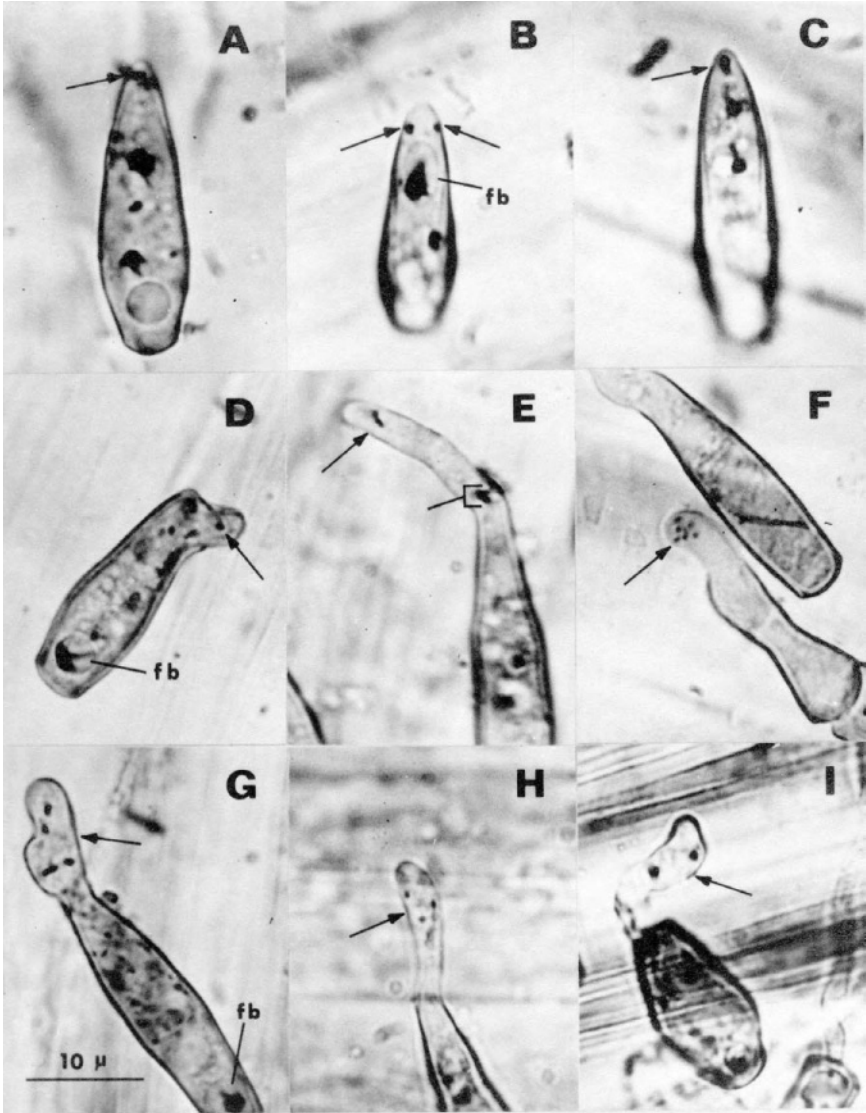
Fig. 6. Esterase-positive reaction sites in conidia, germinated conidia, and appressoria of *Venturia inaequalis*. A and B: pregermination stage of development. Arrows point to crystals of indigo blue. Fat body with crystals of indigo blue (fb). C: Initial stage of germination. Crystal of indigo blue at the tip of the emerging germ tube. D: Initial stage of germination. Crystals of indigo blue in the emerging germ tube and in fat bodies. E: Crystals of indigo blue near the tip of the emerging germ tube and where the tube bends. F-I: Crystals of indigo blue in appressoria (arrows).

2. Management of apple scab

V. inaequalis infects the herbaceous tissues of its host, and produces spots that reduce production of photosynthate and degrade the value of fruit (Aylor, 1998; Jones and Aldwinckle, 1990). Infected leaves are shed each year and the fungus overwinters in the fallen leaves. Primary infections are caused by ascospores that originate from the overwintered leaves and are released in early spring to coincide with the appearance of new floral and leaf tissues (Fig. 7). Initial (primary) infection can lead to production of conidia on infected tissues within 9 to 17 days, and these asexual spores can cause numerous secondary infections. Several waves of secondary infection can occur during a single growing season. Complete crop loss can result and severe infection can reduce blossom bud formation and crop potential for the following year, which may promote biennial bearing. Scab management is an essential component of orchard management in climates that are conducive to infection.

Apple leaf and fruit tissues are most susceptible to infection when they are young and expanding. Thus, scab is most severe where rain, and thus leaf wetness, is common during the early part of the growing season. Some of the main areas of commercial apple production in the world are situated in arid climates and escape scab. Other areas are not so fortunate, and aggressive disease management is required to control the disease.

2.1. Breaking the disease cycle

The disease cycle of apple scab offers several opportunities for control (Fig. 7). Cultivars of apples with resistance to *V. inaequalis* are available. Removal of fallen leaves prior to the beginning of the growing season eliminates sources of primary inoculum. Preventing sporulation by the fungus in fallen leaves would have the same result. Infection requires leaf wetness, which can be avoided by growing apples in dry climates or by providing tree or row covers. Infection can also be prevented with protectant fungicides or antagonistic leaf microflora. Eradicant fungicides can stop development of the fungus in established infections, or prevent production of spores in established infections.
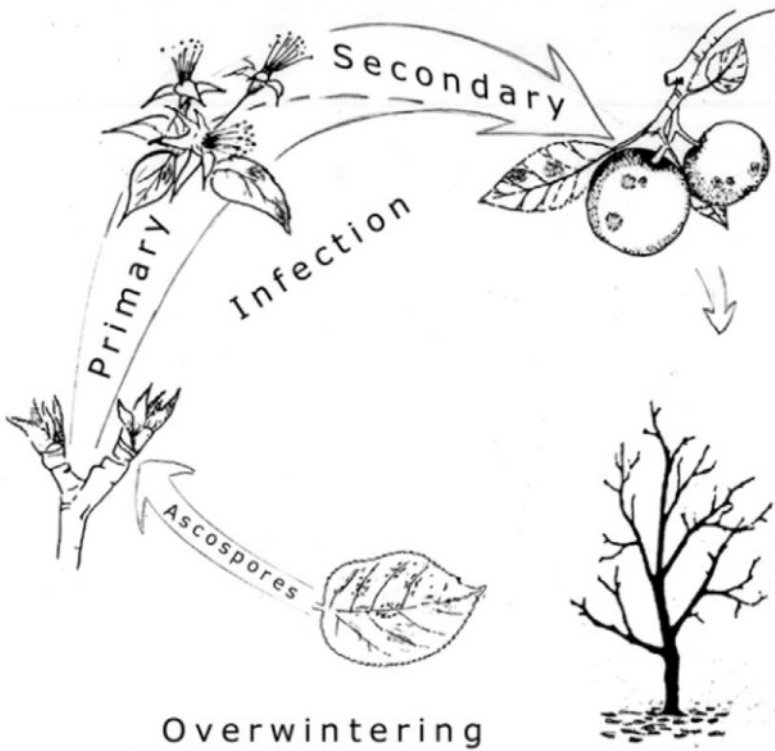
Fig. 7. Opportunities for control of the apple scab disease are especially evident in the primary and secondary stages of infection. In these stages, control is through the use of fungicides, either eradicants or protectants. Control by sanitation during the over wintering stage is also effective.

Destruction of sources of primary inoculum can contribute significantly to an integrated program of scab management, but when used alone has not given sufficient levels of control to meet the standards of the commercial apple industry. Scab can be completely controlled with fungicides where the climate favors disease. It can also be controlled by the use of scab resistant cultivars. At present, growers of scab resistant varieties of apples must create their own markets, since none of the scab resistant cultivars has achieved commercial market share. Control of scab in commercial apple production relies heavily on the use of fungicides (MacHardy, 1996).

2.2. Managing apple scab with fungicides

Fungicides that are currently available for control of apple scab can be categorized as either protectant or eradicant in nature (Table 1). Protectant fungicides adhere to external surfaces and need not be absorbed into plant tissues to be effective. When leaf wetness occurs,

TABLE 1

Characteristics of some fungicides used for control of apple scab

Chemistry	Examples	Affects	Activity	Use	Resistance
benzimidazoles and benzimidazole precursors	benomyl, thiophanate methyl	DNA synthesis	single site	protectant, eradicant	yes, no longer used (Jones and Walker, 1976, Sholberg, *et al.* 1989)
Diverse chemistries (Copping and Hewitt, 1998, Uesugi, Y. 1998)	Nova, Rally, Rubigan, Nustar	sterol biosynthesis	single site	eradicant	yes (Hildebrand *et al*, 1988, Stanis, and Jones, 1985)
strobilurins (Clough and Godfrey 1998)	Flint, Sovran	mitochondrial electron transport	single site	eradicant	at risk (Copping, and Hewitt, 1998)
anilinopyrimidines	cyprodinil	Enzyme secretion; methionine biosynthesis	Single site?	eradicant	?
aliphatic guanidine	dodine	sterol demethylation?	single site?	protectant, eradicant	yes, (Jones and Walker 1976, Sholberg, *et al.* 1989)
Sulfur		mitochondrial electron transport	multi site	protectant	no
phtahlimide	captan, captafol	multiple enzyme inhibition	multi site	protectant	no
dithiocarbamates	mancozeb, metiram, thiram, zineb	enzyme inhibition, at sulfhydryl groups	multi site	protectant	no

a small proportion of the adhering protectant enters into solution. If the amount in the soluble phase is of sufficient concentration and activity, it can redistribute on the leaf surface and block spore germination and penetration. The efficacy of protectants on plant surfaces declines exponentially following application due to weathering and breakdown caused by sunlight, and by expansion of the leaf surface area that is associated with leaf growth. Protectants that do not enter plant tissues have little effect on established infections. To be effective, protectants must be applied prior to, or within 12-24 hours of the start of an infection period, and be maintained in effective concentrations on the surfaces of herbaceous tissues for as long as primary inoculum is available and weather and host development favor infection. In climates conducive to disease, protectants may have to be applied at 7-10 day intervals for as long as 3 months following the green tip stage of bud development.

Eradicant fungicides are absorbed and translocated to varying degrees within plant tissues, and thus have potential to affect development of the pathogen in infections that have already become established. Eradicant fungicides have considerable 'reach back' activity, which means that they are generally able to arrest infections if applied within 96 hours after the beginning of an infection period. Some eradicants act by blocking spore production and reducing the potential of established infections to serve as sources of secondary inoculum for new infections. Fungicides that are absorbed into plant tissues must target specific aspects of fungal metabolism that are not components of plant metabolism. Because of their specific mode of action, eradicants impose strong selection pressures for resistance in target fungus populations. When initially introduced, eradicants are typically very effective, often at very low rates of application. In practice, eradicants that have been used extensively for control of apple scab have selected for resistance within 2-4 years and then have had to be replaced by new eradicants with different modes of action. Because protectants do not enter plant tissues, they can affect a wide range of metabolic processes without being phytotoxic. Most protectant fungicides have retained their efficacy for four or more decades of extensive use.

2.3. Rationale for chemical control

The basis of effective chemical control is prevention of primary infection. Once primary infection occurs and sources of secondary inoculum become abundant, even repeated applications of fungicides at high frequency cannot prevent the occurrence of additional secondary infections.

The efficiency of fungicide use for control of apple scab can be increased in some climates by use of disease prediction models. *V. inaequalis* requires free water in order to infect. The duration of leaf wetness required for infection is inversely related to temperature, and ranges from 41 hours at 0.1^OC to 6 hours at $17-24^O$C (MacHardy, 1989). The relationship between leaf wetness and temperature is the basis of disease prediction models that indicate when infection periods have occurred, and signal the need for timely application of fungicide. In climates that have relatively few infection periods, disease prediction systems have the potential to prevent application of unnecessary sprays and thus reduce the overall number of fungicide applications. Eradicant fungicides are most useful in control programs where disease prediction systems based on weather monitoring are used to indicate the occurrence of infection periods. The extended reach back activity of eradicants usually provides ample time for their application after an infection period has occurred. Prediction systems are of little value in climates where infection periods frequently occur at intervals of less than 10 days during the first 2-3 months of the growing season and thus demand continuous protection of leaf and fruit surfaces.

Because of the propensity of eradicant fungicides to select for resistance, they should be used with limited frequency and in rotation with other fungicides with different modes of action. Eradicant fungicides are most valuable in areas where infection periods are rare, and where prediction systems have the greatest utility. Routine use of eradicants is inappropriate in areas where frequent infection periods require that regular applications of fungicide be made to prevent the occurrence of primary infection.

There is a trend to argue that protectant fungicides with broad-spectrum activity pose greater risks than do eradicants with selective activity. This view ignores the fact that eradicants, when used

extensively, typically remain effective for only a few years, whereas protectants such as captan and dithiocarbamates have been used for 40-50 years and have remained effective. The key analysis should be comparison of the known low risks posed by captan and the dithiocarbamates with the unknown risks posed by a predictable succession of eradicants lacking chronic use history, a succession that may be accelerated in the absence of protectants. The labels of most eradicant fungicides in current use state that these products should be used in combination or rotation with a protectant fungicide to reduce the probability of selection for resistance to the eradicant fungicide.

2.4. Destruction of sources of primary inoculum

Decreased use of pesticides occurs as public attitudes and policies make alternatives economically competitive. Organic production, and production based on integrated pest management and integrated crop production practices are gaining market share. Management of apple scab with reduced dependency on fungicides will require increased emphasis on practices that attack the overwintering phase of the disease cycle. Two such approaches are enhanced leaf litter decomposition and the use of hyperparasites that attack *V. inaequalis* during the overwintering phase of the disease.

Efforts to use sanitation, leaf litter decomposition and eradication of *V. inaequalis* in overwintering leaves as a means of reducing ascospore release and primary infection are reviewed in Sutton *et al.* (2000). These authors also provide original data on the effect of urea sprays applied at 95% leaf fall and in the spring prior to bud break, alone and in combination with shredding leaf litter with a flail mower. Both sanitation and urea reduced ascospore release by 50% - 97% in 17 of 20 trials. Incidence of fruit with apple scab lesions was reduced by 31% to 75% in 11 of 12 trials. The authors conclude that reduction of overwintering apple leaf litter has the potential to reduce the amount of fungicide needed to control apple scab in areas where winters are mild and moist.

Use of biological agents to attack *V. inaequalis* in overwintering leaves is reviewed in Carisse *et al.* (2000). The authors also evaluated the efficacy of six fungal antagonists applied in the fall for reducing ascospore discharge by *V. inaequalis* the following spring. A fungus

originally isolated from apple leaf litter (Bernier *et al.* 1996) and subsequently described as *Microsphaeropsis ochracea* sp. nov. (Carisse and Bernier 2002), was the most effective of these fungal antagonists. Reductions in ascospore discharge averaged 71% and 80% in 1997 and 1998, for applications made after harvest, either just before or just after leaf fall. *M. ochracea* is a saphrophyte on senescent plant tissue but can also parasitize fungal mycelium and the pseudothecia of *V. inaequalis* (Benyagoub *et al*. 1998).

Efforts are currently underway to develop a commercial biological control agent with *Microsphaeropsis ochracea* (P130A) as the active principle. Many biocontrol studies suffer from the use of excessively high levels of inoculum, leading to the false hope for commercialization of biological antagonists. *Athelia bombacina* inhibited all pseudothecial development in the field when a very high antagonist population was used (Gupta 1979), but efficacy dropped to 60-70% when a lower rate was used (Miedtke and Kennel 1990). Understanding the rate effect and early cooperation with an industrial partner has led to the use of realistic inoculum levels throughout the development of *M. ochracea* (P130A). Field results with these low inoculum levels have been promising. Philom Bios in Saskatoon Saskatchewan and Engage Agro in Guelph Ontario are working together to develop *M. ochracea* to have it available as a registered product in Canada and the United States within 3-5 years.

3.   Looking to the future

Scab resistant apple cultivars, and attack on *V. inaequalis* in overwintering leaves are practical and effective options for scab control by home gardeners and for niche marketers, but have thus far had little impact in the North American commercial apple industry. This could change with increasing restrictions on the use of fungicides for control of apple scab. Transfer of Vf or other genes that confer resistance to *V. inaequalis* directly into commercial apple cultivars and the availability and acceptance of such genetically engineered cultivars would likely be followed by acceptance in the commercial industry. Vf resistance currently provides field immunity against apple

scab in most areas of North America. If use of the Vf gene were to become common practice in commercial production, selection for virulence against Vf resistance would likely occur within a decade or two. This has been the experience nin certain areas of Europe where Vf cultivars have been grown more extensively than in North America (Parisi *et al.*, 1993). Scab on Vf cultivars has also been reported in North America (Rahe, 1997). Some apple breeding programs have as their objective the development of durable resistance to *V. inaequalis* through combination of Vf and general resistance to apple scab (Quamme, *et al.*, 2002).

The profitability of commercial apple production in North America has become increasingly marginal during the past two decades. Recent major increases in apple production in China have reduced the US share of export markets in Southeast Asia from more than 50% to less than 25% in the past 6 years, and this trend continues. In North America, the diversity and significance of niche production increases as profitability for mainline commercial production declines. Niche production opens the door to a wider range of tactics that can be used to weaken the apple scab disease cycle. Scab resistance, leaf litter management, use of hyperparasites of *V. inaequalis* and other forms of biological control are likely to become increasingly important components of the overall management of apple scab in commercial apple production in future years.

Economics of commercial apple production in North America have become increasingly bleak during the past two decades. Major increases in apple production in China have occurred, and have reduced the US share of export markets in Southeast Asia from more than 50% to less than 25% in the past 6 years, and this trend continues. As prospects for mainline commercial producers become less attractive, the diversity and significance of niche producers increases. Niche production opens the door to a wide range of tactics that can be used to weaken the apple scab disease cycle. Scab resistance, leaf litter management, use of hyperparasites of *V. inaequalis* and other forms of biological control are likely to become increasingly important components of the overall management of apple scab in commercial apple production in future years.

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3

State of the Art and Challenges of Post-harvest Disease Management in Apples

M.H. Jijakli and P. Lepoivre

ABSTRACT: Despite modern storage facilities, losses from 5 to 25% of apples are still being recorded in storage room. Fungal pathogens such as *Botrytis cinerea*, *Penicillium expansum* and *Gloeosporides* group are mainly responsible of important economical losses even if physiological disorders (bitter pit, water core and storage scald) cannot be neglected. Post-harvest disease control is a complex problem which cannot be solved by a single solution. The control of factors affecting the fruit physiology with pre- and post-harvest handling practices, the sanitation and the application of synthetic fungicides in pre- and post-harvest treatments are the primary means of controlling post-harvest diseases. However, the future use of fungicides is uncertain due to the development of pathogen resistance, the consumer reluctance to chemical residues in food and environment and the consequent growing scarcity of fungicides aimed at post-harvest situations. Several novel approaches (including biological control agents, natural biocides and induction of fruit defence mechanisms) are emerging as possible alternatives to synthetic fungicides. However, the complete replacement of the chemical pesticides by one of these alternative methods is unrealistic because of their lack of efficacy in case of high disease pressure. These alternative methods must be integrated in association with limited quantities of fungicides, as well as efficient management and handling practices to combat diseases in harvested apples. This novel IPM approach should be completed by further studies on predictive models of post-harvest disease development and genetic resistance.

1. Introduction

Apple trees belong to the family of Rosaceae. These fruit trees are grouped under the name *Malus* x *domestica* Borkh. (Bondoux, 1992). However, the origins of the actual cultivated varieties are complex and remain uncertain (Jones and Aldwinckle, 1990). In 2000, the apple production reached around 60 millions tons in the world (FAOSTAT, 2002). The same year, Western European apples production (European

Union 15 countries + Switzerland + Norway) was the first worldwide producer with 9.64 millions tons of harvested fruits whilst Eastern European production was of about 3.50 millions tons. USA constitutes another major producer of apples (4.8 millions tons). Brazil, Argentine, Chile, South Africa, New Zeeland and Australia are also important apple growers with 1.16, 0.83, 0.75, 0.65, 0.48 and 0.33 millions tons of fruits, respectively.

The surface extension of orchards since 1950 was partially due to the improving of storage methods. These methods allowed to extend the life period of harvested apples and to spread out their commercialization. Despite these modern storage facilities, post-harvest diseases of apple annually cause losses of 5-25 %, since early 1970s (Bondoux, 1992). Accurate data on the scale of losses are difficult to obtain and, where fungicidal treatments are applied either before or after harvest, only indicate the incidence of those species which survive such treatment. The most accurate surveys were made in the 1960s (Edney, 1983). Nevertheless, fungal pathogens are responsible of important economical losses even if physiological disorders cannot be neglected.

Until now, post-harvest diseases of apples are largely controlled by pre- and post-harvest handling practices and the application of synthetic fungicides. However, the possible deregistration of effective and widely used fungicides (Wellings, 1996), the development of fungicide-resistant strains of post-harvest pathogens (Franclet, 1994) and the increase of Integrated Pest Management (IPM) (or Integrated Fruit Production, IFP) and organic culture in the context of sustainable agriculture (Cross, 2000) increased the demand to develop alternative methods to control diseases. That need is strengthend by consumer reluctance to chemical residues in food and public concern for environmental safety. Several novel approaches are emerging as alternatives to synthetic fungicides. The purpose of this work is to describe principal post-harvest diseases and present conventional and emerging methods for controlling post-harvest diseases of apples.

2.   Fungal diseases

The importance of each fungal pathogen can vary from one country to another. In Belgium and France (Bondoux, 1992) most losses are

attributable to *Penicillium expansum* Link, *Botrytis cinerea* Pers., and the Gloeosporides group. In USA and UK, *B. cinerea* and *P. expansum*, *Pezicula malicorticis* (H. Jacks.) Nannfs. and *Mucor piriformis* E. Fischer are the most important agents of post harvest diseases (Rosenberger, 1991). For practical reason, the classification of fruit diseases due to fungal pathogens is based on their mode of penetration in the fruit and their further evolution (Table 1).

TABLE 1

Characterisation of common postharvest fungal diseases

	Disease name	Causal agent	Source of contamination	Incidence
Lenticel rot[1]	Bitter rot	*Colletotrichum gloeosporioides*[2]	Cankers	Economical incidence worldwide
	Bull's eye rot	*Cryptosporiopsis curvispora*[2]	Cankers	Economical incidence particularly in Pacific Northwest and in Europe
	Gloeosporium rot	*Trichoseptoria fructigena*[2]	Cankers and leaves	Economical incidence particularly in Europe
Core rot[1]	Mouldy core rot and dry core rot	*Alternaria* spp.	Dry organs	Local and/or sporadic incidence
Eye rot[1]	Dry eye rot	*Botrytis cinerea*[2]	Various debris	Local incidence in North America, Europe and New Zealand
Wound pathogens	Blue mould	*Penicillium* spp	Various debris	Economical incidence worldwide
	Grey mould	*Botrytis cinerea*[2]	Various debris and cankers	Economical incidence worldwide
	Brown rot	*Monilinia fructicola*[2] and *M. fructigena*[2]	Mummified fruit and cankers	Economical incidence in North America, New Zealand and Australia (M *fructicola*) and in Europe (*M. fructigena*)
Other alterations	Mucor rot	*Mucor piriformis*	Organic matter	Economical incidence in USA
	Phytophtora rot	*Phytophthora syringae* and *P. cactorum*[2]	Soil and cankers	Sporadic incidence in Europe

1: latent infections, 2: species able to infect other organs than fruits

2.1. Latent infection

Post-harvest decay attributable to latent pathogens results from infections that occur in the field but remain quiescent and escape notice at harvest.

2.1.1.   Lenticel rot

Various fungal species are able to infect fruits through lenticels. These species belong to the "Gloeosporides" group and constitute one of the major source of post-harvest apple losses.

"Bitter" rot is caused by *Colletotrichum gloeosporioides* (Penz.) Sacc. (syn. *Gloeosporium fructigenum* Berk.). The teleomorph is *Glomerella cingulata* Stoneman. Bitter rot is a common disease of apples in practically all countries where they are commercially grown (Fig. 1). Lesions originating from infections by conidial types (which produce only conidia) are circular and become sunken when they enlarge (Jones and Aldwinckle, 1990). Acervuli are produced in concentric circles around the infection point. Lesions initiated by perithecial types (which produce both ascospores and conidia) are darker brown and not sunken. Conidial masses associated with these perithecial types are first orange-salmon in early stage and turn to dark brown. Fruit infection can occur before, during or just after bloom. Fruit are equally susceptible during all stages of development (Bondoux, 1992). At the optimum temperature (26°C), latent infection can occur with a wet period as short as 5 hours (Koelher, 2000).

Fig. 1:   Biter rot (provided by P. Creemers, Royal Research Station of Gorsem, Belgium)

The causal agent of "bull's eye rot" is *Cryptosporiopsis curvispora* (Peck) Gremmen (syn. *Gloeosporium perennans* Zell. et Childs). The perfect stage is *Pezicula malicorticis* [syn. *Cryptosporiopsis malicorticis* (Cordl.) Nannf.]. Bull's eye rot seems to be an important disease in the Pacific Northwest and in Europe. It is more effective parasite of woody tissue and was initially described as a "perennial canker" (Edney, 1983). *C. curvispora* causes circular spots around the lenticels which grow slowly. Lesions appear slightly sunken and brown with a lighter brown centre. Numerous lesions can occur on the same fruit and are originated from different lenticels (Creemers, 1998). Conidia are produced in acervuli throughout the year and dispersed by rain. Fruit can become infected anytime between petal fall and harvest. Fruit susceptibility increases as the growing season progresses (Jones and Aldwinckle, 1990).

"Gloeosporium rot" is due to *Trichoseptoria fructigena* Maubl. [syn. *Gloeosporium album* Osterwalder, teleomorph *Pezicula alba* (Grunthr.)]. This species is an important parasite of dessert apples, particularly in Europe. *T. fructigena* is very similar to *C. curvispora* in terms of symptoms and biology. Nevertheless, it parasitises wood with difficulty and is found mainly on dead wood (Edney, 1983). As the conidia are produced throughout the year, fruit may become contaminated at any time during the growing season.

Other species can provoke occasionally and/or locally some losses may be due to fungi such as *Cylindrocarpon mali* (All.) Wr., *Alternaria* spp., *Stemphylium botryosum* Wallr. and *Cladosporium herbarum* Lk. (Bondoux, 1992).

2.1.2. Core rot

Most cultivars susceptible to core rot (*i.e.* 'Gloster', 'Belle de Boskoop'…) have an open sinus extending from the calyx into the core (Creemers, 1998). Mouldy core may develop into dry core rot if the pathogen penetrates into the core flesh. But the fungus is generally limited to the core or carpel region (Jones and Aldwinckle, 1990). External symptoms are rare, except infected fruit may colour and fall prematurely. Several fungi [such as *Alternaria* spp., *Stemphylium* spp., *Cladosporium* spp., *Phomopsis mali*, *Fusarium avenaceum* (Fr.) Sacc., *Trichothecium roseum* (Bull.) Lk.] can be associated with mouldy core and dry core rot (Bondoux, 1992 ; Creemers, 1998; Jones and Aldwinckle, 1990).

2.1.3. Eye rot and calyx end rot

Dry eye rot or blossom-end rot has been reported on apples in North America, Europe and New Zealand (Jones and Aldwinckle, 1990). The first symptoms (red discoloration) appear at the base of one or more of the sepals on the calyx end of the fruit. Dry-eye rot evolves as a shallow, hard rot over a small area often with a red border. These alterations are often due to *Botrytis cinerea* but other species (*Cylindrocarpon mali* and *Alternaria* spp.) can also provoke similar symptoms.

Nectria galligena Bres. is the causal agent of "Nectria canker" that can kill young trees and branches of older trees, can also infect apple fruit resulting in an eye rot disease. This eye rot is characterised by slightly depressed, brown necrotic areas on the fruit surface (Jones and Aldwinckle, 1990).

Calyx end rot is a sporadic and minor disease of apple fruit which is characterised by a soft rot. It may expand to cover about 1/3 of the end of a fruit.

It is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary (Jones and Aldwinckle, 1990 and Koelher, 2000).

2.2. Wound pathogens

All the post-harvest pathogens on apples are potential wound pathogens. In practical conditions, only some fungi which are able to fast growing are responsible for important apple loss (Bondoux, 1992). When fruit has been weakened by ripening and aging, the infection speed is comparable to growth on an artificial medium (Creemers, 1998). These fungal agents infect fruits by deposition of airborne or waterborne conidia on wounds during harvesting, transport and handling before storage (Jijakli *et al.*, 1999). The higher frequency of apple wounds with the mechanisation of the harvest and conditioning processes before storage explains the increased importance of wounds pathogens.

At least 11 species have been isolated from naturally infected pome fruits exhibiting "blue mould" symptoms, the most frequent being *P. expansum*, *P. solitum* Westling and *P. commune* Thom (Jones and Aldwinckle, 1990). The species that causes the most extensive decay is *P. expansum* (Fig. 2). Most infection caused by *Penicillium sp*. is

Fig. 2:   Blue mould (provided by P. Creemers Royal Research Station of Gorsem, Belgium)

initiated at wound sites, such as cuts and stem punctures, but fruit can also become infected through lenticels on unbroken skin, particularly at bruise sites. Typical symptoms of blue mould are circular, tan coloured lesions with sharp margins between the watery soft rot and healthy fruit flesh (Bondoux, 1992). Production of blue-green spores can occur on the surface of the decay. They form a dense, powdery mass at the centre of the lesion (Jones and Aldwinckle, 1990). *Penicillium* spp. can be isolated from orchard soils, but the disease is rare in the field except on fruit that have fallen to the ground. Airborne conidia originating from decayed fruit or from sporulation on bins and storage walls are present in packinghouses and storages.

"Grey Mould" (*Botrytis cinerea*) is also a common worldwide decay of apples (Fig. 3). Grey mould lesions are characterised by pale tan mouldy areas without sharp margins, older portions of the decay turning darker brown (Bondoux, 1992). Darker spots around the lenticels may appear on some varieties. Sporulating mycelium is gray, but little sporulation occurs at cold-storage temperatures (Jijakli and Lepoivre, 1998). Black sclerotia can be produced on fruit, especially around the infection site. The source of *Botrytis* spores is the orchard. The fungus grows and sporulates abundantly on dead and dying plant

Fig. 3: Grey mould (provided by P. Creemers, Royal Research Station of Gorsem, Belgium)

material found in orchard cover crops, especially during cool, moist weather (Jones and Aldwinckle, 1990). These initially rotted fruits spread the disease through fruit contact to produce nests of decaying fruit.

Three species of *Monilia* cause "brown rot" of apple (Fig. 4). *M. fructicola* (Wint.) Honey is established throughout North America, New Zealand and Australia and attacks injured apples as they ripen in some occasions (*i.e.* a buildup of the disease on neighbouring stone fruit crops). This pathogen has recently been isolated in France from stone fruits but not yet from pome fruits (Lichou *et al*., 2002). *M. fructigena* Pers. is the most common species in Europe and is considered as a major disease in that geographical area (Jones and Aldwinckle, 1990). The third species, *M. laxa* (Ehrenb.) Sacc., is rare on apple on which it can cause fruit rot. Superficial, circular brown spots expand outward on the surface of the fruit and result in a soft decay of the flesh. Spots of gray-white fungus may develop on the surface of the lesions and are arranged in concentric band (Jones and Aldwinckle, 1990). *M. fructigena* overwinters in infected peduncles or twig cankers on branches and produces conidia which are disseminated by rain and infect blossoms. Conidia, produced on infected blossom and twigs, infect wounded fruit as they mature.

Fig. 4:   Brown rot (provided by P. Creemers Royal Research Station of Gorsem, Belgium)

2.3. Other post-harvest fungal diseases

"Mucor rot", caused primarily by *Mucor piriformis*, is less frequently encountered than *B. cinerea* and *Penicillium spp.*, but can be highly destructive (Sanderson, 2000). Losses due to this disease have been serious in United States. Lesions are watery with less distinct margins than those in fruit with blue mould (Jones and Aldwinckle, 1990). Fruit are relatively quickly decayed with the entire fruit often involved so that little is left of the fruit a few months after infection occurs. Similar to gray mould, mycelia are often present on the surface of diseased fruit. They are water and insect disseminated and can contaminate packinghouse water systems.

"Coprinus rot" (*Coprinus psychromorbidus* Redhead and Traquair) has been found throughout the Pacific Northwest. It is often mistaken for bull's eye rot. Fruit infection occurs during the last month before harvest. This fungus appears as a white, cobwebby growth on the surface of infected fruit and will create nest or cluster rot like gray mould (Kupferman, 1993).

"Alternaria rot" [*Alternaria alternata* (Fr.) Keissler] may occur on apples in any production stage (Kupferman, 1993). This fungus lives on dead and decaying plant tissue in the orchard. Spores contaminate fruits in the orchard and during the handling process. The amount of decay depends on the condition of the fruit. Infection usually occurs through breaks in the skin or other weakened areas caused by sunburn, bruising, chemical injury or scald.

"Phytophtora rot" is caused by *Phytophthora syringae* (Klebahn) Klebahn and *P. cactorum* (Lebert and Cohn) Schröter. It is usually of sporadic occurrence and of limited economical importance (Creemers, 1998). Nevertheless, this disease provoked important economical losses in several European countries in the 1970's due to high humid conditions. Rotted fruits are typically marbled olive green or brown to uniformly pale brown in apple. Rotted flesh has a alcoholic odour and vascular tissue are dark-stained. Both pathogens perennate as oospores in apple orchard soils. Fruits rot epidemics are associated with high rainfall in cool weather for *P. syringae* and in warm weather for *P. cactorum*. Inoculum is splashed onto fruit and infection occurs *via* the lenticels.

3.   Physiological disorders

A number of disorders in fruit are known as physiological because they are not the result of damage by micro-organisms or insects. The physiological disorders (Table 2) can be influenced by environmental, horticultural, or biological factors. For example, bitter pit and water core are correlated with orchard factors (water and mineral nutrition) while other disorders (storage scald, Jonathan spot) are more often considered as storage accidents (Bondoux, 1992). Nevertheless, the origin of some physiological alterations is often difficult to know because symptoms can be associated to different causes. For this reason, it is difficult to classify them.

TABLE 2
Characterisation of common physiological disorders
(modified from Bondoux *et al*. 1992)

Disorder	Cause	Correlation with mineral content	Period of apparition
Bitter pit	Cellular collapses	Ca (-), Mg (+), K (+), P (+), N (+)	Orchard and storage
Water core	Cell wall rupture (excess of glycerol)	Ca (-), N (+)	Orchard and beginning of storage
Storage Scald	Production of toxic compounds	Ca (-), N (+)	Storage
Russet	Abnormal growth of the epidermal cells	N (+)	Storage
Jonathan spot	Cellular collapses	Ca (-)	Storage
Deep browning	Senescence, low temperature injury and gas concentration during storage	Ca (-)	Storage

"Bitter pit" usually appears as depressed brown lesions in the skin of fruits (Fig. 5), located mainly on the calyx end of the fruit (Ferguson and Watkins, 1989). Peeling the affected area reveals dry, brown corky flesh. It often appears after harvest, although it can be found in fruits of certain varieties in the orchard when the problem is

Fig. 5: Bitter pit (provided by P. Creemers, Royal Research Station of Gorsem, Belgium)

severe. Bitter pit is a disorder of apples related to a mineral imbalance within the fruit (Retamales and Valdes, 2000). The incidence of the disorder is related to a deficiency of Ca content in the fruit and, in general, is directly related to magnesium, potassium, phosphorous and nitrogen levels in fruit tissues. Numerous other factors have been associated with bitter pit such as genetic predisposition, fruit size and cropping status, canopy attributes, rootstocks, irrigation and water status, fruit developmental rate and maturity, storage conditions.

"Water core" consists in liquid-soaked tissue mainly around the vascular bundles (Loescher and Kupferman, 1985). Nevertheless, the disorder can also appear in any part of the flesh tissue. In case of severe attack, the tissues turn to a glassy appearance due to the presence of liquid in the intercellular spaces. Analytical comparisons of affected and healthy fruits have shown elevated water content, decreased reducing sugars and higher sorbitol content in water-cored apples. Water core tissue lacks the ability to convert sorbitol to fructose creating the accumulation of toxic compounds such as ethanol and acetaldehyde (Jones and Aldwinckle, 1990). High nitrogen and low calcium fruit concentration can increase the incidence of the disorder. As bitter pit, symptoms can appear in the orchard.

"Storage scald" (or "common scald" or "superficial scald") is a physiological disorder characterized by a brown discoloration of the skin (Kupferman, 1993) (Fig. 6). Only a few layers of cells beneath the skin are affected. Usually, fruit develop scald symptoms after storage when they are exposed at room temperature during a few days. The production of naturally toxic compounds (terpene, α-farnesene) in the fruit peel seems to cause this browning (Jones and Aldwinckle, 1990). Factors influencing the increase of storage scald severity are early harvest, high nitrogen and low calcium fruit content, warm pre-harvest weather, delayed cold storage, high temperature and relative humidity in storage room.

Fig. 6:   Storage scald (provided by P. Creemers Royal Research Station of Gorsem, Belgium)

"Russet" symptoms are characterised by cork on the outer surface of fruit (Jones and Aldwinckle, 1990). Apple russet is associated with some environmental conditions, such as high humidity, rain or dew on the fruit, frost. Russet disorder is related to abnormal growth of the epidermal cells. Physical damage of the cuticle (particularly between bloom and 30 days after petal fall) can stimulate too rapid division of underlying epidermal cells, causing the cuticle rupture followed by the cork development. Other factors are also associated with the

disorder: improper nutrition (*i.e.* high nitrogen), harsh chemicals, or infection by *Pseudomonas* bacteria.

"Jonathan spot" is often associated with lenticels. Symptoms begin with small brown to black spots (Jones and Aldwinckle, 1990). As the disorder progress, spots coalesce and form irregularly shaped blotches. The spots usually do not penetrate the flesh. The disorder can appear on other cultivars than 'Jonathan' (*i.e.* 'Golden Delicious', 'Idared', 'Newtown'…). The occurrence of Jonathan spot is related with low calcium concentration and storage procedures (slow cooling, high storage temperature).

Finally, "deep browning" of the apple flesh can also appear (Jones and Aldwinckle, 1990). Symptoms are generally characterised by various tanning but some other alterations may be present (cracking, floury fruit,…). These symptoms can be attribute to overmaturity of the fruit, low temperature injury and atmosphere composition (too high concentration of CO_2 eventually linked to a low O_2 concentration).

4.   Traditional methods of control

4.1. Control of factors influencing the fruit physiology

A number of practices linked to the control of fruit physiology are designed to prevent or to delay the incidence of post-harvest diseases in apples. These include controlling growth conditions of trees in the orchard, harvesting before the climacteric rise, avoiding mechanical injuries and modifying the environment during pre-storage, storage and transit in order to reduce the rate of respiration.

Strategies for decay prevention must include proper fertilization. As described before, incidence of several physiological disorders can be attributed to improper nutrition (high nitrogen and/or low calcium concentration). For example, bitter pit being linked to the calcium concentration of fruit tissues, its control has been achieved mainly toward supplementing the Ca supply to the fruit *via* pre-harvest foliar applications eventually followed by a post-harvest calcium treatment by dipping, drenching or pressure infiltration (Conway, 1991 ; Conway and Sams, 1983).

Incorrect nutrition, as provided by high nitrogen, makes also fruit more susceptible to some fungal decays. Studies by Sugar (1994) showed that decay was reduced in fruit with relatively low nitrogen to calcium ratios. On the other hand, fruits presenting a high potassium/calcium ratio reach more rapidly the climacteric point and become subsequently more susceptible to fungal decay. Beyond this indirect effect, higher calcium concentrations in apples inhibit or delay the symptom development by *P. expansum*, *B. cinerea* or *C. gloeosporioides* (Conway, 1991). It was demonstrated that higher calcium content in apple tissue improves the cell wall structure and integrity (Conway, 1987). It was also observed that the inhibition of some pathogens such as *P. expansum*. by Ca was linked to the decreased of polygalacturonase activity produced by the pathogen. To spray trees with calcium chloride during the growing season is thus also recommended to delay fungal decay in stored fruit. In that context, management of the orchard must provide fruit at harvest with high and balanced mineral nutrient contents to have a low risk of disorders and have optimal storage properties.

The evolution of fruit maturity plays an important role in the development of rots. Apples must be harvested before the climacteric rise. Pre-climacteric fruits are usually firmer than mature fruits and more resistant to mechanical injuries occurring during harvest and post-harvest handling before storage. Moreover, the internal resistance of fruits against fungal diseases decreases with maturity (Creemers, 1998).

It is crucial to operate carefully during harvest and post-harvest handling in order to limit mechanical injuries (Herregods, 1990). The ability of wounds to heal plays an important role in the resistance against wound pathogens and is associated to the ethylene production (Sommer, 1989). In apples, as for many others fruits, the healing process is characterized by the construction of a periderm. The generation of that barrier is possible during cell division and extension. After harvest, the fruit is not able anymore to produce such a barrier. Nevertheless, cells around the wound site are able to strengthen their wall by synthesising molecules based on lignin and callose. The optimal conditions to promote such a process (85 % RH and 10°C) are rarely met during harvest and post-harvest treatment and never during storage. In contrary to this, when apples are received in the

packinghouse, they should be placed into cold storage so that field heat can be removed as quickly as possible. On unwounded fruits, rapid removal of heat has a positive effect on both fruit quality and reduction in storage decay. Room loading and bin stacking procedures should be established to allow the rapid filling of rooms, and excellent air flow for cooling (Kupferman, 1986).

Storage environmental conditions of apples such as moisture, ventilation, temperature and oxygen and carbon dioxide concentrations directly influence the development of physiological disorders (cf. 3) and fungal diseases. High relative humidity and low temperatures were first applied respectively to avoid fruit desiccation and to delay the maturation but these conditions were not enough efficient to control the fruit respiration. Actually, technical progresses allow storing the fruits in controlled atmosphere (CA). Such CA storage rooms are characterized by a low oxygen concentration (2 to 3 %) and a high carbon dioxide content (2 to 5 %). The deprivation of oxygen is supported until a level of 2 % (or sometime less) by numerous varieties (Kupferman, 2001). Atmospheres containing simultaneously very low O_2 (1 to 1.5 %) and CO_2 levels are also employed. These ultra low oxygen (ULO) storage rooms slow down the respiration process and ethylene synthesis, insuring the firmness of the fruits and increasing the storage period of 7 to 9 months (Marcellin, 1990). Novel storage rooms are developed in United-Kingdom, Italy and USA (Kupferman, 2000) where the ethylene produced by apples is eliminated. An increase of acidity, flavour and firmness of 'McIntosh', 'Empire' and 'Golden Delicious' was observed when these varieties were stored in ULO with low ethylene level in comparison with classical ULO. However, it is impossible until now to maintain an extremely low level of ethylene in the storage atmosphere when the volatile compound is produced in high quantity (Kupferman, 2000).

The phytosanitary problems have evolved in parallel with the technical changes of storage. Some physiological disorders are associated to CA or ULO conservation. For example, low oxygen can sometimes induce a loss of flavour followed by an alcoholic flavour generated by anaerobic fermentation. In some varieties, the red area of the skin will turn purple and green areas into bronze. The sensitivity to high level of carbon dioxide is more frequent and linked to the variety, the fruit age and the origin of harvest (Kupferman, 2001;

Marcelin, 1990). The skin of the fruit will be rough and stained with a swonflake pattern on some varieties. The flesh of affected fruit will become brown and in many cases will develop cavities. The core tissue may also turn brown (coreflush). 'Cox's Orange', 'Granny Smith' and 'Elstar' are more sensitive to high CO_2 level than 'Golden Delicious' and 'Jonagold'. In modified atmosphere associated with the control of ethylene level, a decrease of scald was observed on 'Golden Delicious' (Kupferman, 2000).

Low temperature decreases the growth of fungal pathogens. The critical temperature inhibiting the fungal growth depends on the pathogen. Nevertheless, fungal diseases responsible of important economical losses (*B. cinerea*, *P. expansum* and *C. curvispora*) are able to growth at 0°C, apples being stored at slightly higher temperature. CA and ULO conditions frequently inhibit the sporulation of fungi. Fungal growth can be directly decreased in ULO storage. Due to the physiological preservation of apples, the development of latent infections is sometimes sufficiently delayed to allow the commercialisation of the fruits before the occurrence of symptoms. High relative humidity in the storage rooms favours the development of pathogens (Creemers, 1998). Furthermore, poor ventilation around storage containers leads to increased moisture around the fruit and slower cooling times, which can increase the risk of infection.

4.2. Sanitation

Sanitation is a useful tool in post-harvest disease management strategy and consists in reducing, removing, eliminating, or destroying inoculum at the source. In case of post-harvest apples systems, this includes sanitation of field bins, packinghouse water systems, and packing and storage facilities to avoid inoculum of fungal post-harvest pathogens.

Part of the sanitation strategy consists in discarding damaged fruit lying around a packinghouse. A single apple may have billions of fungal spores on its surface that may be redistributed to new infection sites (Kupferman, 1986). It has also been recognized that spore of fungal pathogens accumulate in water systems (Heald *et al*., 1928). Dump tank water sanitation is critical to reduce spore loads in water because the higher the numbers of spores in dump tank water system, the more infection sites will be inoculated and the greater the risk of decay (Spotts,

1986). The contribution of contaminated field bins to populations in drenches and flume water systems has been established more recently, showing the necessity to also sanitise that material (Sanderson, 2000). Spores produced on decay lesions on fruit in storage can be blown around the rooms by the refrigeration fans and may cause new infection sites. These spores, in addition to those that persist on fruit surfaces and bins, also can contaminate water systems. For the same reason, packing facilities and surfaces must also be sanitised.

In many countries, there is no systematic process for sanitizing field bins even if numerous techniques can be used such as washing with a disinfectant (chlorine, sodium orthophenylphenate or SOPP, quaternary ammonia compounds), pressure washing with hot water, or steam cleaning (Apel, 1989; Kupferman, 1986). Chlorine compounds, quaternary ammonia formulations, and steam are also effective sanitizers for packinghouse line/hard surfaces, including cold storage room surfaces. Chlorine is a biocide also used to treat process water (Apel, 1993). Different salts of chlorine (sodium hypochlorite, calcium hypochlorite, bromochlorodimethyl-hydantoin) are recommended depending on the country legislation. Chlorine should only be used in solutions where the pH (acidity/alkalinity) is around neutral (pH 6.5-7.5) (Kupeferman, 1986). The biocide activity of these salts is also directly influenced by the temperature, the concentration of the product (50 to 100 ppm of free chlorine), and the amount of dirt in the water (Kupeferman, 1986; Eckert and Ogawa, 1988).

Ozone can also take part of an overall sanitation program to disinfect cold storage rooms and water systems (Tukey, 1993). Ozone, a very powerful oxidizer, can be generated through the use of ultraviolet (UV) light or electricity. The latter method, called corona discharge, is the most common way to generate ozone in large quantities. Ozone presents the advantage to have a short half-life time (15 minutes). For this reason, it needs thorough mixing to be effective as a water disinfectant treatment. Disadvantages of its use are its corrosive activity to many common materials, like rubber and mild steel and its human toxic effect.

In the mid 1990's, studies showed that acetic acid vapour was very effective in killing fungal spores of post-harvest pathogens (Sholberg and Gaunce, 1995). These results indicated that acetic acid

vapour could be a possible alternative, or could be used in conjunction with chlorine for disinfecting fruit.

4.3. Chemical treatments against post-harvest diseases

Until now, control measures against post-harvest diseases are mainly based on the protection of fruits from pre- and post-harvest infection with pre- and post-harvest treatments. These treatments aim at depositing enough quantity of active ingredients on fruits to insure their protection against diseases during the total period of storage.

For pre-harvest treatments, the strategy consists in several applications of fungicides to prevent post-harvest fungal diseases. The active ingredients used against post-harvest pathogens belong to benzimidazole or MBC (benomyl, carbendazim, thiophanate-methyl, thiabendazole or TBZ), phenylcarbamate (diethofencarb), phtalimide (captan), dithiocarbamate (thiram, ziram), sulfamide (tolylfluanide) (Creemers, 1998; Eckert and Ogawa, 1988; Environmental Protection Agency, 2002; Franclet, 1994; Jijakli *et al.*, 1999; Locke *et al.*, 2002; Sanderson, 2000). The number of active ingredients may vary per country depending on its legislation. Examples are presented in Table 3.

TABLE 3
Registered conventional active ingredients for pre-harvest
applications in Belgium, France, UK and USA

Fungicide group	Active ingredient	Diseases (partially) controlled * against major diseases	Countries
Benzimidazole	Benomyl	B, P and G	USA
(MBC)	Carbendazim	B, P and G	Belgium, UK and USA
	Thiabendazole	B, P and G	Belgium, USA
	Thiophanate methyl	B, P and G	Belgium, France, UK, and USA
Dithiocarbamate	Thiram	B, P and G	Belgium, UK and USA
	Ziram	B, P and G	USA
Phtalimide	Captan	B and G	Belgium, UK, and USA
Sulfamide	Tolyfluanide	B	Belgium and France

* : Expected control in absence of resistant strains of the pathogen

B = *B. cinerea*, P = *Penicillium* spp., G. = *Gloeosporium* spp.

The number of pre-harvest fungicidal treatments against post-harvest diseases ranges generally around 3 to 4 in orchards presenting a high density of pathogens (*B. cinerea*, *Gloeosporium* spp. and *Penicillium* spp.). For example, six weeks before harvest, a benzimidazole fungicide is applied in Belgium, followed by a treatment with captan or thiram, an application of benzimidazole 14 days before picking, then comes a last spraying with tolyfluanid one week before harvest (Creemers, 1998). In France, the most usual programme of treatments includes two sprays with tolyfluanide and one application of an active ingredient belonging to the benzimidazoles.

Post-harvest treatments are applied to control both physiological disorders and fungal diseases occurring after harvest. When apple varieties such as 'Delicious' are highly susceptible to the physiological disorders (storage scald), it is recommended to treat them with an antioxidant, either diphenylamine (DPA) or ethoxyquin (Sanderson, 2000 ; Biggs and Rosenberger, 2001).

The number of active ingredients authorized for post-harvest applications may also varies from one country to another (*see* Table 4). The sole active ingredient registered in France is TBZ. Currently,

TABLE 4

Registered conventional active ingredients for post-harvest applications in Australia, Argentina, Belgium, France, UK and USA

Fungicide group	Active ingredient	Diseases (partially) Countries	controlled *
Benzimidazole (MBC)	Benomyl	B, P and G	Argentina,
	Carbendazim	B, P and G	Australia, Argentina, and UK
	Thiabendazole	B, P and G	Australia, Argentina, Belgium, France, and USA
	Thiophanate methyl	B, P and G	Argentina and UK
Dithiocarbamate	Iprodione	B, P and G	Argentina and Australia
Imidazole	Imazalil	B and P	Argentina and Belgium
Phenylamide + MBC	Metalaxyl + carbendazim	B and G	UK
Phtalimide	Captan	B, P and G	UK and USA

* : Expected control in absence of resistant strains of the pathogen
B = *B. cinerea*, P = *Penicillium* spp., G. = *Gloeosporium* spp.

only two conventional post-harvest fungicides (captan and TBZ) are allowed for use on apples in the United States (Sanderson, 2000). Many other fruit producing countries can use fungicides that are not registered in the United States or in France. In Australia, the benzimidazole fungicides (carbendazim and TBZ) and iprodione are registered. Benomyl, TBZ, carbendazim, thiophanate methyl, as well as iprodione and imazalil are authorised for post-harvest application in Argentina (Sanderson, 2000). Other examples are listed in Table 4. In that frame, fruits from one country should be segregated and treated for the export market to which the fruit is assigned, as it is already applied in both Argentina and Chile.

Benzimidazole family is mainly used against *B. cinerea*. These fungicides are applied since 1970 (Eckert and Ogawa, 1988). Certain strains of *Penicillium* or *Botrytis* are resistant to all the benzimidazole family (Biggs and Rosenberger, 2001). This phenomenon was foreseeable due to the intensive use of these fungicides against the same pathogens on other crops (Creemers, 1987). The first resistant strains were detected in late 1970's in storage rooms. The resistance seems to be persistent when no benzimidazole is applied anymore. Despite the restriction of their use against post-harvest diseases and their interdiction against scab or powdery mildew in some countries, resistant strains of *B. cinerea* and *Penicillium* sp. are still present in most cold chambers (Franclet, 1994; Prusky *et al*., 1985 ; Rosenberger, 1991; Vinas *et al*., 1991). Recent work revealed that between 30% to 50% of isolates of *P. expansum*, recovered from drenches, were resistant to TBZ (Sanderson, 2000). Furthermore, growing number of *Gloeosporium* spp. resistant strains to benzimidazole fungicides appeared during the last years (Bondoux, 1992). In the mid-1980s, most of the fungicide resistant strains of *Penicillium* and *B. cinerea* were unusually sensitive to DPA (Biggs and Rosenberger, 2001). Thus, the combination of DPA and a benzimidazole fungicide provide good control against both pathogens though the late 1980's. However, about 2 % of the *P. expansum* strains recovered from apple storages were resistant to both DPA and TBZ, even in the mid-1980's. It appears that these strains have gradually increased in importance and should be at least partially responsible for declining effectiveness of post-harvest treatments in some countries (Biggs and Rosenberger, 2001).

In order to avoid the development of fungicide-resistant strains, the anti-resistance strategies during pre- and/or post-harvest treatments should consist in alternating unisite fungicide family with different modes of action and associate multisite fungicides such as tolyfluanide, thiram or captan (Creemers, 1998). In USA, one current approach for controlling TBZ-resistant strains of *Pencillium* is to add captan to the post-harvest treatment solutions (Biggs and Rosenberger, 2001). Tolyfluanid has a large spectrum of action and its use for pre-harvest application is advised in an anti-resistance strategy in France and in Belgium (Creemers, 1998). On the other hand, a negative cross-resistance is usually seen between the fungicide families benzimidazole and phenylcarbamate. Sumico, a product based on carbendazim and diethofencarb and commercialised in Belgium, is used to acquire a predominant role in the control of post-harvest diseases of apples (Creemers, 1998). However, this product is not registered anymore in Belgium.

Indeed, governmental policies of several countries are restricting the use of fungicides or are reassessing the registration dossier of widely used molecules (Gullino and Kuijpers, 1994; Ragdsdale and Sisler, 1994; Wellings, 1996). Because countries are currently revising the (re)registration of active ingredients, Tables 3 and 4 should be used as a general reference. The use of vinchlozoline is already restricted during the flowering period in Europe. Application of captan is not allowed anymore in Germany, while the period between its last application in the orchard and the harvest was enlarged in other European countries. The possible deregistration of benzimidazole family is also discussed at European community level. Benomyl is already forbidden and the carbendazim deregistration could follow. In USA, some fungicides have already lost their post-harvest registrations, such as benomyl and iprodione. Four commonly used post-harvest chemicals (captan, TBZ but also DPA and SOPP) were recently scheduled for a tolerance reassessment by the U.S. Environmental Protection Agency (EPA) (Warner, 1998). An additional ten-fold safety factor may be applied for chemicals used on foods that are common in the diets of infants and children. If captan and DPA were reregistered, the current status of the other molecules remains unclear. EPA will reassess other active ingredients in the near future.

Apples being considered as a minor crop for agro-chemical companies and the registration process being expensive in comparison of the potential market size, there is no specific development of novel fungicides against post-harvest diseases on apples. However, novel synthetic fungicides, which were recently developed against diseases on major crops, are being evaluated against apple rot agents (Sanderson, 2000). Trifloxystrobin, belonging to the strobilurin family, is already registered in Switzerland for pre-harvest treatments against Gloeosporiodes group. But the pre-harvest use of trifloxystrobin in Europe will be probably limited because the main targeted pathogens are apple scab and mildew and resistance phenomenons were already detected for both fungi (Creemers, personal communication). Fludioxonil (pyrrole), fenhexamid (anilide), tebuconazole (triazole) and cyprodinil (anilino-pyrimidine) are being tested in several European countries and USA. Fludioxonil has shown broad spectrum efficacy against post-harvest pathogens, whereas fenhexamid is very effective against *B. cinerea*, *Monilia* spp. but not against *Penicillium* spp. or *Gloeosporium* spp. (Sanderson, 2000). Tebuconazole and cyprodinil are also efficient against *B. cinerea*. Some of these active ingredients are already registered to treat other fruits than apples against storage diseases but the authorisation of their use against post-harvest diseases on apples might take a couple of years and it is still not clear if they will be used for pre- or post-harvest treatments.

5.   Integrated control and organic production

The consumer reluctance to chemical residues in food and the public concern for human and environmental safety have promoted both the restrictions of fungicide uses and the emergence of Integrated Fruit Production (IFP) and organic orchards.

The approach to Integrated Plant Protection in sustainable production system was described by Boller *et al*. (1999). All available prophylactic (indirect) plant protection measures must be applied before direct control measures are used. The decision of the application of direct control measures must be based on economic thresholds, and risk assessments. Priority is given to natural, cultural, biological,

genetic, and biotechnical methods of disease control and the use of agrochemicals must be minimized (Cross, 2002). The IFP market has considerably increased this last decade and reached 53% of the surface of European countries/regions in 1997 (Dickler *et al.*, 1999). The producers of IFP are allowed to treat their orchards with a limited number of products classified according to their efficacy, selectivity and environmental safety (Cross, 2002). Until now, the Integrated Plant Protection concept was mainly developed with success for the protection against insects and pre-harvest fungal pathogens such as apple scab or powdery mildew. However, the IFP guideline imposes that post-harvest fungicide treatments may only be used where suitable non-chemical methods are not available. Furthermore, post-harvest treatment with synthetic, non-naturally occurring anti-oxidants for control of superficial scald and other disorders is not allowed in Europe. The search and the development of alternatives methods for the control of post-harvest diseases is an important challenge for the further development of this integrated approach. Furthermore, post-harvest treatments are authorized only on fruits with a high probability of rotting. Unfortunately, only few predictive models have been published such as a model for bitter pit risk on Golden (Sio *et al.*, 2001) or a model for rot risk on Cox in England (Berrie, 2000).

The organic production market remains a marginal sector of activity (less than 3 % in Europe and in USA). No treatment against post-harvest rots is applied in European organic orchards. Authorized fungicidal treatments (copper and sulfur-based products) are only active against scab. Copper could be also deregistered in Europe in 2004 due to its toxicity on the soil fauna. As a consequence, the persistence of this organic sector relies on the finding of new control methods.

6.   Emerging technologies of control

The progressive loss of fungicide effectiveness due to selection of resistant isolates of pathogens, the growing scarcity of fungicides devoted at post-harvest situations and the public pressure concerning the risk of residues on fruits have promoted the search for alternative methods. Several novel approaches are emerging as possible

alternatives to synthetic fungicides, including biological control agents (BCAs), application of natural biocides, induction of natural defence mechanisms of harvested products, and genetic resistance (Falik *et al.*, 1995; Janiziewicz and Korsten, 2002; Jijakli *et al.*, 1999; Tukey, 1993 ; Wilson *et al.*, 1994).

6.1. Biological control

Biological control is generating a great enthusiasm to play a role in sustainable agriculture although the relevance of BCAs in plant pathology appears limited until now. If everybody recognises the existence of natural phenomena of microbial antagonism, the question is to know how to manipulate the occurring antagonistic micro-organisms to achieve a reliable and effective strategy of disease control meeting the requirements of the market. The post-harvest phase is particularly suited for the application of biological control methods (Jijakli *et al.*, 1999). The application sites are limited to the fruits and the harvested commodities are of high value. Furthermore, variation of temperature, relative humidity, or gas composition can be minimized in storage room allowing the selection of micro-organisms better suited for one set of particular conditions.

Before becoming an economically feasible alternative to chemical control, BCAs have to satisfy different requirements related to biological, technological and toxicological properties.

The different steps of research and development for a successful strategy of disease control with BCAs are represented in Fig. 7. These steps are all essential and complementary to the others. An "ideal antagonist" should have the following characteristics (Jijakli *et al.*, 1999): effective at low concentrations in several post-harvest host pathogen combinations; able to survive under adverse environmental conditions such as low temperatures and controlled atmospheres prevailing in storage facilities; amenable to inexpensive production and formulation with a long shelf life; easy to dispense; compatible with commercial handling practices; genetically stable; non pathogenic for the consumer and for the host commodity.

There are numerous examples in the literature of biocontrol agents, active against wound pathogens (mainly *B. cinerea* and *P.*
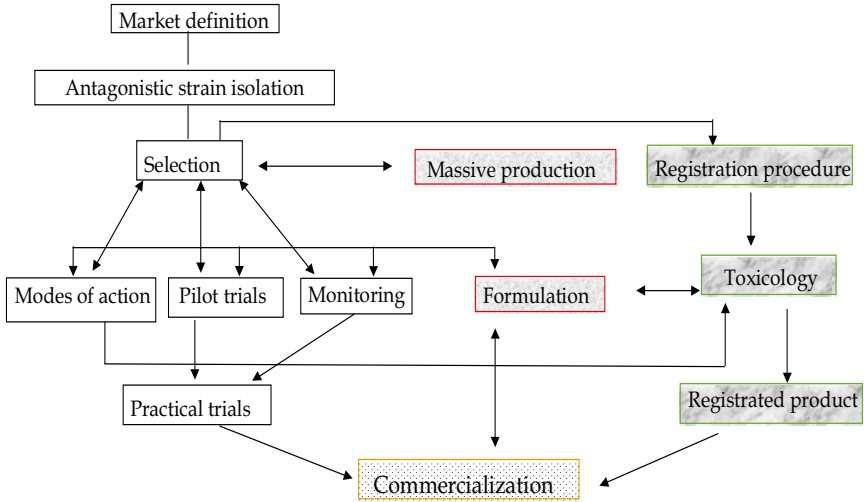
Fig. 7:   Steps leading to the practical use of BCA's (modified from Jijakli *et al.,*
1999)

expansum) of apple fruits (for a review *see* Janisiewicz and Kosten,
2002; Jijakli *et al*., 1999; Wilson and Wisnieswski, 1994), but to date
only three have reached the market. Two biocontrol products have
been commercialised in USA and are used as post-harvest treatments
on apples for control of wound diseases: Biosave™ (*Pseudomonas
syringae*, Esc-11) by Ecoscience Corp and Aspire™ (*Candida
oleophila*, I-182) by Ecogen Inc. Yield Plus™ (*Cryptoccoccus albidus*)
constitutes the third biocontrol product against post harvest diseases
on pome fruits and is sold in South Africa by Anchor Yeast, but little
has been reported about that last product (Janisiewicz, and Kosten,
2002). In Europe, no biocontrol products active against post-harvest
diseases of apples are available until now. However, some biological
control agents such as *Candida oleophila* strain O (Jijakli *et al*., 1999)
or *Candida sake* strain CPA-1 (Usall *et al*., 2000) are in the
development phase and might reach the European market soon. There
are several bottlenecks explaining the low number of registered
biopesticides for use on apples against post-harvest diseases and the
moderate success of such commercialised products. Among them,
the lack of reproducibility and reliability of BCAs efficacy when they
are used in practice constitutes the major limiting factor.

The improvement of the biocontrol has been accomplished by
several approaches such as (i) the combination of several BCAs against

a same pathogen (Nunes *et al*., 2002), sometimes on basis of niche differentiation (Janisiewicz, 1996); (ii) the enlargement of the spectrum of controlled diseases with mixtures of compatibles BCAs (Janisiewicz 1988; Janisiewicz and Bors, 1995) or selection of particular micro-organisms controlling a panel of pathogens (Janisiewicz *et al*., 1996); (iii) the manipulation of the environment in which the BCA will operate such as the addition of nutrients (Janisiewicz, 1994; Jijakli *et al*., 1999); (iv) the combining of the BCA treatment with low doses of fungicides (Chandgoyal and Spotts, 1996), organic (El-Ghaouth *et al*., 2000, Jijakli *et al*., 2003) and inorganic additives (Jijakli *et al*., 1999; Nunes *et al*., 2002), or physical treatments (Leverentz *et al*., 2000; Jijakli *et al*., 1999); (v) the suitable production and formulation of the antagonists (waxes, salts,….) (Abadias *et al*., 2001); (vi) the physiological improvement of the BCA's (Texido *et al*., 1998).

More recently, pre-harvest treatments with BCAs were also assessed against post-harvest wound pathogens on fruits with some success (Ippolito and Nigro, 2000 ; Jijakli *et al*., 2003). Antagonists are applied few days before harvest in order to precolonize the fruit surface. It is expected that antagonistic strain will colonize the wounds created during harvest prior pathogen colonisation. In that approach, many of the advantages of post-harvest application are lost (Janisiewicz, and Kosten, 2002) and environmental fate studies must be undertaken to assess the ecological suitability of a particular strain under unfavourable factors like UV light, changes in temperature, humidity and nutrient availability, *etc*. (Jijakli *et al*., 2003).

The science and practice of biological control agents is still in its infancy compared to fungicidal treatment, even if the progress made in this area during the past decade and a half has been remarkable (Janisiewicz, and Kosten, 2002). In the long term, basic information on the genetically determined factors that control survival, colonisation, effectiveness in the field and storage and properties of mass production are required to overcome the random process of selection and to facilitate the practical development of such a method (Jijakli *et al*., 1999). This information will help in finding how to (i) enhance the protective action of BCAs, (ii) protect the viability and the performance of BCAs under unfavourable environmental conditions, (iii) ensure a

good stability of the product during storage prior to application, and (iv) provide a user-friendly product that is easy to apply.

6.2. Natural biocides

Plants produce a large number of compounds (constitutively or after induction) with potential activity against micro-organisms. Among these compounds, some extracts and essential oils from various plants revealed a biocide action. Recently, large *in vitro* studies on the control of fruits post-harvest pathogens have been carried out (Daferera *et al*. 2000; Wilson *et al*., 1997) and showed fungicidal or fungistatic activity. Three essential oils produced by *Cymbopogon martinii*, *Thymus zygis* and *Eugenia caryophyllata* were the most efficient inhibitors of *in vitro B. cinerea* spore germination (Wilson *et al*., 1997). Thymol and citral, two essential oils, showed *in vitro* a high inhibitory effect against *P. expansum* growth (Venturini *et al*., 2002). The use of carvone (mint extract) or eugenol (clove extract) by dipping harvested apples controlled the development of post-harvest fungal pathogens on these fruits (Bompeix *et al*., 2000). However, the high concentrations required to obtain an acceptable protective level could constitute an economical barrier for practical application.

Some volatile aromatic components produced by fruit during ripening such as acetaldehyde also showed fungicidal or fungistatic activity. The resistance of strawberries to some pathogens is partially attributed to a high production of acetaldehyde (Wilson and Wisniewski, 1989). Some volatile compounds present a low toxicity for mammals (*see* Mari and Guizzardi, 1998 for a review) and might be promising in fumigation in cold storage or in packaging.

Other natural products are derived from other organisms. Chitosan is a high-molecular weight polysaccharide derived from alkaline deacetylation of chitin, an animal component (de Capdeville *et al*., 2002). Chitosan controlled partially the development of post-harvest decays such as *B. cinerea* or *P. expansum* (de Capdeville *et al*., 2002). This action is the result of direct toxic effect on pathogens, indirect effect on fruit senescence and stimulation of fruit resistance by increasing chitinase and β-1,3-glucanase activities (de Capdeville *et al*., 2002).

6.3. Intensification of natural defence mechanisms

Fruits contain a multitude of highly coordinated defensive mechanisms that naturally protect them from invading micro-organisms (Forbes-Smith, 1999). Accumulation of phytoalexins, modification of the structural barriers, and synthesis of antifungal hydrolases such as chitinase and β-1,3-glucanase are part of the various natural plant defence strategies against microbial attack that should be enhanced. This objective can primarily be attained by slowing down the ripening process (*i.e.* by manipulating storage conditions, *see* 4.1) in order to maintain constitutive and inducible defence responses. Further induction of resistance against post-harvest rots has also been observed after physical, chemical or biological treatments.

Physical methods of control of post-harvest diseases on apples are promising because they leave no residues in/on the fruit. The beneficial effect of low doses of UV-c occurs in several post-harvest commodities including stone, pome and citrus fruit (Stevens *et al*., 1996). The development of disease resistance after such a treatment coincides with the accumulation of phytoalexins in host tissue such as lemon, carrot roots or grapes (Forbes-Smith, 1999). In grapefruit, UV-c light treatment is correlated with an increase of phenylalanine ammonia-lyase and peroxidase activities (Droby *et al*., 1993). Wilson *et al*. (1997) developed an apparatus that delivers UV-c light. Its application on a processing line significantly reduces post-harvest decay in apples. UV-c light also directly affects the pathogen as shown on conidial survival of *P. expansum* (Valdebenito-Sanhueza and Maia, 2001). The optimal dosage of UV-c light depends on cultivars and physiological age (Forbes-Smith, 1999). Application of too high doses may increase the susceptibility of the host tissue to pathogen invasion.

Pre-storage heating of post-harvest commodities may be also advantageous as a natural control strategy by directly inhibiting pathogen growth (Jijakli *et al*., 1999) but also by accelerating natural resistance of host tissue. In case of apples, the stimulation of wound healing process seems to be partially responsible for the resistance against post-harvest diseases. Thermal treatments (bath at 45°C, 10 min) were susceptible of reducing latent infections (Jijakli *et al*., 1999) and disinfect the surface of the fruits but does not offer a long-term

protection (microbiological vacuum). However, this treatment may increase the susceptibility to *P. expansum*, *B. cinerea* or *Alternaria* sp. (Edney and Burchill, 1967, Jijakli *et al*., 1999).

Several chemical compounds such as chitosan (cf. 6.2), calcium (cf. 4.1), harpin or acibenzolar have shown their ability to induce resistance in harvested apple fruit. For example, harpin, a peptide produced by the plant pathogenic bacterium, *Erwinia amylovora* (Burrill) Winslow *et al*., induced resistance in apple fruit against blue mould and the resistance depended on harpin concentration and the interval between treatment and inoculation (Capdeville *et al*., 2002). Nevertheless, the protective level due to these compounds doesn't reach the level of conventional fungicides. Finally, the possibility to induce resistance has also been observed with antagonistic yeast's on apples infected by *B. cinerea* or *P. expansum* (Capdeville *et al*., 2002; El Ghaouth *et al*., 2000).

6.4. Genetic resistance

Spotts *et al.* (1999) have recently shown differences among apple varieties through a study of their susceptibility to four post-harvest fungal pathogens and through some physical properties (force to break epidermis, sinus opening). Natural sources of resistance can then be found, but selection programs are time-consuming.

Insights into genes involved in ripening, senescence, defence reactions, respiration, as well as into expression of factors triggering genes after harvest or at stage where the host is more sensitive, lead to good hope of developing resistant plants by traditional breeding or genetic transformation (*see* Arul, 1994, for a review). For example, considerable progress has been made on ethylene regulating fruit ripening in association with its perception and signal transduction and gene expression (Jiang and Fu, 2000). ACC synthase and ACC oxidase, two proteins involved in the ethylene regulation, have been characterized and their genes cloned from various fruit tissues. The properties and functions of ethylene receptors are also being elucidated. As the apple sensitivity to post-harvest diseases is partially linked to fruit physiology, the prospects of ethylene regulating fruit ripening associated with post-harvest life extension should be promising.

The use of foreign genes are also envisaged and gave mitigated results in the following example (pre-harvest field): a line of transgenic apple tree overexpressing an endochitianse (CHIT42) form *Trichoderma harzianum* Rifai proved more resistant to *Venturia inaequalis* (Cook) Wint. but showed a reduced vigor (Bolar *et al*., 2000). Transgenic broccoli expressing the same enzyme showed reduced sensitivity to *Alternaria brassicola* (Schweinitz) Wiltshire (Mora and Earle, 2001). However, little attention has been paid until now to apple post-harvest diseases.

7.   Conclusions

After analysing the current status of post-harvest disease control, it is evident that there is no single solution to such a complex problem. The control of factors affecting the fruit physiology with orchard operations and post-harvest handling practices, the sanitation and the application of synthetic fungicides in pre- and post-harvest treatments are the primary means of controlling post-harvest diseases for conventional IPM programs. However, the presence of chemical residues in food, the development of fungicide-resistant strains of post-harvest pathogens, the deregistration of standard fungicides have generated interest in the development of alternative methods.

Some alternative methods such as natural biocides, BCAs, physical or chemical treatments to induce fruit defence mechanisms, seem promising. BCAs are particularly suited for post-harvest applications against wound pathogens and some of them are already available on the US market. However, the review of emerging methods including physical, chemical and biological treatments demonstrate that in many situation and primarily when the disease pressure is relatively high, the level of protection by the use of a single alternative method rarely reached the one obtained by conventional synthetic fungicide application (if the problem of fungicidal resistance is absent). The complete replacement of the chemical pesticides by one alternative method is then not a reasonable goal. In contrary to this, each method has to be considered as a tool to be used in integrated control strategies. A more realistic scenario would see alternative techniques being used in association with limited quantities of agro-chemicals, as well as

efficient management and handling practices to combat diseases in harvested apples. This novel IPM approach must take into account the compatibility of the different treatments, particularly between antagonistic micro-organisms and chemical/physical techniques. That more complex approach is already evaluated in different countries (Biggs *et al*., 2000; Habib *et al*., 2001) and will probably emerge as novel IPM recommendations.

Finally, more attention should be paid to the genetic resistance approach and to the development of reliable forecast systems for post-harvest diseases. The choice of an IPM program should be selected in relation with the predicted disease pressure. The use of synthetic fungicides might be avoided in case of low pathogen pressure. These further studies will help in obtaining a global integrated control strategy to manage post-harvest diseases on apples.

8.   References

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4

Role of Vertebrates in Inflicting Diseases in Fruit Orchards and their Management

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ABSTRACT: Documented information on the inter-relationships of the fruit tree, the disease causing agent and the vertebrate is exceedingly fragmentary. Animals cause wounds for the pathogenic organisms to gain entry and cause disease. They also disperse disease causing agents, actively or passively. The phanerogamic plant parasites, *viz.,* species of *Loranthus* and *Dendrophthae* on mango trees is of common occurrence in India, dispersed by birds particularly Thickbilled flowerpeckers (*Dicaeum agile* Tickell).

About fifty species of vertebrates are implicated in causing damage to horticultural crops in India. Only a few, however, incur economic losses or serve as effective agents of pathogenic organisms. The same species of vertebrate, notably Roseringed parakeet, *Psittacula krameri* Bechstein*,* Wildboar, *Sus scrofa* W*,* squirrels of species of *Funambulus*, rats of species of *Rattus*; bandicoots, *Bandicoota bengalensis* B and *Bandicota indica* B damage several fruit orchards. Even these animals have ecological roles to play in orchards. For instance, wild upturns sub-surface soil to surface; *Funambulus* species squirrels forage on weed seeds and crop pests. These animals also regulate micro vegetation. Orchards in or around wild areas are prone to bison (*Bibos gaurus* H) and elephant (*Elephas maximus* Linn) raids. Most crop raids, however, are incidental resulting from man-animal conflicts. Therefore, a 'Balance sheet' of activities of the vertebrates in different orchards is basically important. Crop protection without vertebrate mortality is desirable in most situations.

Timely harvests and clean cultivation, wrapping or covering of fruits, mulching the base of fruit trees, seasonal pruning, shade regulation, animal-proof trenches, polyculture, baiting and provisioning the orchards with alternative foods for the vertebrates are useful management tools. A harmonious blend of these crop protection tools with solar powered fence, repellent pastes of local materials, scaring and public awareness of the role of vertebrates in orchards will promote conservation of natural resources and sustain good quality fruit yields.

Human population pressure and increasing Human-Animal conflicts is making vertebrate-pathogen-fruit orchard interactions develop into an important science.

1.   Introduction

Diseases of fruit crops are important as often extensive and permanent losses are incurred and management of diseases may prove expensive. Diseases of fruit crops are incited by casual agents like fungi and bacteria and wild animals, like mammals, rodents and birds play a part in the dispersal of certain disease causing agents or predispose the crop for the disease. The phanerogamic plant parasites like *Loranthus* and *Dendrophthae* are dispersed mostly through birds and other animals. The birds are attracted by the bright colors of the fruits of *Dendrophthae fulcata,* the most common species reported in India. The pulp of the fruit that is gulped is sticky and thus the seeds are carried from one place to another by birds. Droppings of birds containing seeds also help in the dissemination of the parasite (Singh, 1998). When healthy, green or ripening fruits are injured, both quality and quantity of the harvested crop are reduced. Similar is the case with the diseases of the roots, trunk and branches inflicted by vertebrates. These diseases generally cause debilitation of the tree by disrupting the translocation of substances between roots and shoots, fruits fail to mature properly, yielding many unsized ones.

It is in the intercontinental spread of pathogens that birds' influence has been documented. Birds have occasionally been suspected of transporting fungal spores from one continent to another (Ingold, 1971), on their breasts, tail feathers, feet and beaks. Some pathogens are carried externally by animals, others internally, some passively, others appear to have an active biological association (Chandniwala, 1996). Vertebrates can inflict diseases in fruit orchards by introducing the pathogen into a healthy crop while feeding, foraging, resting or roosting; transmit the pathogen into a healthy crop over considerable distances or spread from tree to tree in a orchard. For instance, Elephants while foraging can trample or topple a tree in a banana or papaya garden or coconut plantation as often observed in hill region of Karnataka, South India and the affected tree may be a source of disease. But continuous observations over a long time are required to establish the cause-and-affect relationship. Nevertheless,Vertebrates that injure fruit trees or be responsible for a disease in a orchard, cannot be branded a pest. Even these animals have important

ecological roles that aid in the sustainable fruit production (Davries, 1926). For example, bats are very important pollinators and seed dispersers in tropical forests through out the world (Cox *et al*., 1991). Rodents maintain and regulate flow of materials between producers and decomposers and detrivores and alter the plant production characters (Hayward and Phillipson, 1979). Secondly, much is known about the spread of several pathogens by man and by insects, but very little about those dispersed by vertebrates. Thirdly, comprehensive knowledge on all aspects of vertebrates is a prerequisite before effective management practices can be evolved (Chakravarthy, 2000; Chakravarthy and Srihari, 2001). The management practices depend mainly on the habitat and habits of the vertebrates which vary widely from one place to another even in the orchards of the same crop, in a climatic zone. Further, the size of the orchard and economics of the farmers also counts. This review embracing pomology, plant pathology and vertebrate science is focused on fruit diseases caused / dispersed by vertebrates, their role and management.

2. Fruit Orchards and Vertebrates

Wild animals use orchards for food, shelter, nesting and roosting, causing often either temporary or permanent damage. The fruit loss due to vertebrate pests is of a great magnitude and little attention is paid to their management (Bose and Mitra, 1980). In India, the diverse agroclimatic conditions permit cultivation of fifty kinds of fruit crops and nuts, many of which are of commercial importance such as mango, banana, citrus and grapes. The area under fruit crops increased during the last two decades, but it is still barely one % of the total cultivated area in the country (Pathak, 1980).

Fruit crops cultivated in or near forest tracts have high vertebrate pressure and face unique problems. This is because such orchards are found in the natural habitat of vertebrates concerned. In such situations, part of requirements of the vertebrates are met by fruit crops. Orchards in the Western ghats, Eastern ghats and Himalayan regions in India have high vertebrate pressure and face greater fruit losses due to vertebrates damage and diseases. Lack of grass and natural foods in the forest is forcing vertebrates to take to fruit crops

which serve as a 'Cafeteria' for wild animals. The secretive and in some species, nocturnal habits of vertebrates and their mobility make it most unlikely that they will actually be seen damaging the fruit crops (Taylor, 1972). Identification and mobility of a vertebrate is a major operational constraint. Much of the damage to orchards is as a result of conversion of or encroachment into, the natural habitats of wild animals.

Orchards in India represent usually a small, mixed crop, biologically diverse ecosystems where sophisticated methods of pest management like chemical applications are not often desirable and not adopted. For instance, 20% of the fruits in apple, pear, peach and apricot orchards in Himachal Pradesh are damaged by birds like Redbilled Blue Magpie (*Cissa erythrorhynchay* Bryth), Redvented Bulbul (*Pycnonotus cafer* Linn.), Whitecheeked Bulbul (*Pycnonotus leucogenys* L.) and Slatyheaded parakeets (*Psittacula himalayana* Lesson). The orchards were successfully protected from bird depredations by repeated sprays of landarin 1.5 kg a.i./ha. However, fruits became unfit for consumption because of toxic residues (Kakar *et al.,* 1986). There is an urgent need to evolve research based management practices that will protect crops and yet conserve the vertebrate species, their ecological functions and natural resources. In order to determine the role of vertebrates in fruit diseases, one has to objectively study habits and assess damage to crops (Puttarudraiah, 1967). So important traits of different groups of vertebrates are briefly given below.

2.1 Bats

Bats are unique among mammals of their size in their long lives, low fecundity, maternal care and slow development (Findley, 1993). Bats are somewhat like birds ecologically. Bats are obviously K-strategists with relatively constant population size, greater competitive ability, iteroparity and greater energetic efficiency.

There are 4200 living species of mammals and almost 1000 of them are bats belonging to the order Chiroptera, sub-order; Megachiroptera (fruit-bats) and Microchiroptera (Insectivorous bats). Of 1000 species, 25% are frugivorous and 88% exclusively tropical. The principal habitat of frugivorous bats include fruit canopy, open

clearings and forest tracts. Droppings from frugivorous bats contain fruit pulp and seeds which may serve as a substrate for disease causing pathogens.

Fruit bats are physiologically and behaviourally adapted for feeding on different kinds of fruits. Mickleburgh *et al.* (1992) recorded *Eidolon helvum* feeding on fruits of 34 plant genera. Similarly *Pteropus* species used flowers of 26 genera and fruits of 62. Fruits of *Ceiba* species attract 11 genera of Megachiroptera and the fruits of *Ficus* sp. atleast thirteen. The food of megachiropteran bats tend to be conspicuous, often clumped and generally abundant and easily harvested within the clumps. This condition may favour rapid multiplication of pathogens among clusters of fruits.

Certain plants play a major role in bat nutrition, the most obvious are the Figs (*Ficus* sp.), a genus of the greatest importance to frugivorous animals throughout the world. Most fruit plants of bats are more synchronous and more seasonal in their production of fruit and we may expect to find a sequential series of flowering and fruiting within a plant assemblage that supports a megachiropteran bat community. Disease causing micro organisms delivered on a flush, by bats serve as a reservoir for the next flush of fruits or the next season.

Bats are very important pollinators and seed dispersers in tropical forests and have shared a long evolutionary history with angiosperms. Megachiropterans feed upon atleast 145 genera of fruits in 30 families of plants ((Mickleburgh *et al*., 1992). The most important families are Palmae (16 genera), Anacardiaceae (10 genera) and Sapotaceae (8 genera). Generally fruits are consumed when ripe, but this is not always so. For example, coconut (*Cocos nucifera* Linn) fruits are eaten when small and immature. It is in this situation that injured fruits form a base for multiplication of disease causing agents. Large fruits as mango (*Mangifera indica* Linn) are consumed *in situ*, but smaller fruits may be carried away from the parent tree before being devoured and the seeds ejected through the mouth or anus. Fruits that are dropped on the way or left partially damaged on the tree, rot.

On many oceanic islands, fruit bats are the only animals capable of carrying large seeded fruits and can be the single, most important pollinators, seed dispersers and 'keystone species' (Cox *et al*., 1991).

Atleast 443 plant products useful to man are derived from 163 plant species that rely to some degree on bats for pollination or seed dispersal (Fujita and Turtle, 1991). For fruit growers beneficial effects of bats outweigh harmful effects (Jacobson and Duplessis, 1976). There is a population decline of bats in recent years. It is for this reason, that maximum protection to fruit crops from bats and birds damage is required. So an action plan for the conservation of fruit bats is of a high priority (Mickleburgh *et al.,* 1992). Because many species of fruit bats are dependent on primary forests and thus threatened by the large scale destruction of forests in tropical areas that bats forage in fruit orchards where they are a cause for damage or disease. The level of fruit damage varies considerably with locality and is generally the maximum in summer when females are lactating and have greater energy requirements (Mickleburgh *et al.*, 1992). Declines in fruit eating bat populations are widespread in India due to high rate of deforestation, increased use of pesticides, habitat degradation and human consumption.

2.2 Birds

There are 8600 species of birds described under 28 orders and 2100 species and sub-species have been found in the Indian subcontinent (Ali and Ripley, 1983), distributed in 20 orders and 33 families, where 350 species are extralimital. Birds are an important component of any ecosystem, occupying and interacting with components at other trophic levels and help in transfer and flow of energy and materials. As a group, birds are major regulators of invertebrates, particularly the dominant animals group, *i.e.* insects. Birds are of value as environmental indicators and usually are top consumers in an ecosystem and so are ecologically and economically of paramount importance (Chakravarthy, 1998).

Birds actively interact in fruit orchards and farmers incur considerable fruit losses and practical solution to their damage are meagre. Roseringed parakeet, *Psittacula krameri* K, House crow, *Corvus splendens* Vieillot. and Jungle crow *Corvus macrorhynchos* Sykes are in general, the fruit depredators, but having beneficial roles in orchards. Growers must protect fruit crops without harming the

birds, utilising insectivory for pest suppression, pollination, seed dispersal, spreading pathogenic microorganisms among pest insects and for sustaining biological diversity. (Chakravarthy and Tejasvi, 1993; Ali and Abdul Ali, 1938; Bhatnagar, 1976). Determination of economical and ecological roles of birds in different fruit orchards and role of birds in human welfare are crucially important.

Most species attack fruits, nuts and seeds than vegetative parts like leaf, branch, stem and roots. Fruits at ripening stage are most vulnerable to bird damage, irrespective of fruit size (Chopra *et al.,* 1972; Chowdhari and Seam, 1996). Therefore, foraging of birds in a fruit tree is dependent on age, than fruit size. Birds descent on the canopy of fruit tree, select a mature fruit because it is easier to extract seeds from ripe than raw fruits (Saini *et al*., 1994). The birds clamber about among the twigs and gnaw into the half-ripe fruits, one after another wasting far more than they actually eat (Ali and Ripley, 1983). The frugivores excavate lumps of pulp of fruit, bit by bit sometimes till the entire pulp is removed. Such fruits are prone to soft rot .Birds have not been described as vectors of plant viruses. But game birds as patridges (*Francolinus pondicerianus* Gmelin) often run between plants before flying and might well be responsible for unaccountable introduction of viruses into healthy plants (Chandniwala, 1996).

Cultivated areas when compared to a number of primary habitats including forests have proved to be more species rich in birds. The need to produce more fruits should not be at the cost of biodiversity. For example, providing perches in fruit orchards, can encourage birds such as drongo, *Dicrurus adsimilis* Vieillot, rollers, *Coracias* sp. that predate on a variety of insects during the day and owls that devour both insects and rodents at night (Mason and Lefroy, 1912). One has to balance the benefits derived from birds against their destructiveness in cultivated areas before any management practices are taken up (Daniels, 1998).

2.3   Rodents and Small Mammals

Rodents, especially rats and their allies, have always been with us, mostly as pests, ever since primitive man became an agriculturist and started having granaries. In Hindu mythology, rats always accompany

the popular diety, the elephant-headed Ganesh the harbinger of prosperity, success and abundance (*"Riddhi, Siddhi, Vriddhi"*), whose blessings are invoked at the start of all functions and religious ceremonies (Roonwal, 1996; Tripathi *et al*., 1999). The Rodentia includes 46 genera, 128 species and 260 subspecies. The six families included are : Sciuridae (Squirrels), Hystricidae (Porcupines), Dipodidae (Jerboss and birch-mice), Muscardinidae (dormice), Rhizomyidae (bamboo-rats) and Muridae (rats, mice, bandicoots, voles and gerbils). The larger account of work done in recent years has been concentrated mostly on rodents of economic importance and their control (Barnett and Prakash, 1975; Prakash and Ghosh, 1985) considerable detail. The majority of the rodent species are herbivorous where they are concern for the cause of plant diseases (Prakash, 1959, 1962, 1968, Prakash and Jain 1970, Prater, 1948).

Rodents are specialised pests of fruit trees and forage on herbs and grasses during their breeding season, using bark or tree roots as a substitute feed during the non-breeding season (Nirula *et al*., 1954). Their breeding habits are also often distinct and sometimes far away from the areas where damage occurs, a feature that makes it difficult to understand and cope with fruit damage (Keshav Bhat *et al.,* 1995). Today in most orchards planting is done by saplings, instead of by seeding. The saplings are produced on a fertilized substrate under plastic or polybag and such saplings are significantly more susceptible to attack by rodents. New and very expensive objects of damage are the grafts in seed orchards (Myllymaki, 1979). Even slight feeding or nibbling or cut, exposes the grafts/saplings to disease causing pathogens.

Lagomorphs include about 90 living species including 29 pikas, 32 hares and 29 rabbits. Lagomorphs are divided into two families, viz., Ochotonidae (pikas) and Leporidae (hares and rabbits). Lagomorphs mostly affect orchards at the roots (soil substrate) of fruit trees or affect seedlings by injuring and exposing the plants to diseases. Lagomorphs inhabit diverse habitats, from deserts to tropical forests and regulate microvegetation in orchards.

Globally there are 2021 species of rodents and a further 428 species of Insectivora. Thus, over 50% of all mammals are members of these two orders. There often exists a misconception that small

mammals are more tolerant to the processes that threaten larger and charismatic mammals with extinction. This is erroneous. Small mammals have the ability to manipulate the population dynamics of different species, exhibit a great variety of responses in their feeding habits, are limited to both the producer and decomposer components and alter plant production characters by suppressing successful reproduction and growth by attacking plant species and mainly affect orchards as a regulator and maintainer of the ecosystem (Hayward and Phillipson, 1979). Rabbits carry viruses on animal's hairs and spread among healthy plans when they brush past infected plants (Chandniwala, 1996).

2.4 Large Mammals

The world spectrum of mammals comprise 4100 species of which 500 are known from India, including 100 species and subspecies inhabiting the vast tracts of the Indian sub continent (Sharma, 1994). Nearly 15% of the assessed mammals are endemic to India (Mollur *et al.,* 1998). Monkeys, *Macaqua radiata* L. Wildboar, *Sus scrofa* and Jungle cat, *Felis chaus* G depredate on fruits in South India. Larger sized animals like bison, *Bibos gaurus* and elephants, *Elephas maximus* raid fruit crops particularly during summer (January-May). In coconut and banana, the animals may damage the entire plants. These two large animals, incidentally or at some instances specifically damage any plant part and predispose the trees / bushes for pathogenic infection. Bison damage to coconut palms result in reduction in palm height and number and size of fronds. Jackal (*Canus aureus* Linn) and Stag (*Cervus canis* Linn) damage ripening pods, break twigs and branches, injure bark by rubbing horns and body against its surface and detops seedlings of fruit plants, particularly cocoa in hill region of Karnataka (Chakarvarthy and Srihari, 2001).

Coconut gardens are also raided by elephants. Observations in hill region of Karnataka during the last decade revealed that maximum damage occurred during July. The damage embraced trampling, browsing, uprooting and detopping of coconut palms (Chakravarthy and Srihari, 2001). These interactions of wild animals predispose the orchard plants to disease causing bioagents.

3. Fruit diseases and Vertebrates

3.1 Apple (*Malus domestica* L)

Narang and Chandel (1995) at Nauni, Solan assessed the extent of damage caused by seven species of birds to apple orchards. The damage was maximal between 0600-0900 h mostly confined to the upper and middle canopy of the tree. Maximum damage to fruits (80%) was caused by Blossom headed Parakeet (*Psittacula cyanocephala* Linn). The damaged fruits were subsequently fed by a number of insect pests and fungi adapted to decaying sweet resources. Such fruits become unfit for marketing. The early maturity compared to late maturing cultivars suffered more bird damage.

Flying foxes (*Pteropus edwardsii* B) caused damage to sapota, papaya, citrus, peach, guava and other fruits in Karnataka. Branches of thorny trees and old fish nets or wire netting protected the fruit bunches. Bats may be deferred to roost near orchards and stored fruits may be damaged due to rodents. Zinc phosphide fumigation helps. Burrow baiting proved superior to surface baiting. All pome fruits like apple, apricot, cherry and bear are prone to collar rot incited by *Phytophthora cactorum*, wounds and injuries caused by animals favour the disease. Wounds and injuries also favour cankers on stem or branch often resulting on death of the bark within the infected area (Pathak, 1980). Apple orchards in India suffer from pink disease, stem black rots and cankers and vertebrates, small as well as big favour development or spread of these diseases.

3.2 Grapes (*Vitis vinifera* Linn)

Bank Mynas (*Acridotheres ginginianus* Latham) and Indian Mynas (*Acridotheres tristis* Linn) damage both immature and mature grapes. Damage is severe when alternate food sources are scarce. More damage occurred in grapevines trained with the Head System than in Bower System (35%). Birds preferred Beauty Seedless Variety of grapes having purple berries (Sandhu and Dhindsa, 1995; Sandhu and Chakravarthy, 1982; Toor and Ramzan, 1974).

In Bangalore, Jungle crow (*Corvus macrorhynchos* Sykes) and House crow (*Corvus splendens* V) are the major pests on grapes.

The Barbet, *Megalaima.viridis* Boddart and three species of bats also incur heavy losses (Prasad and Verghese, 1985). Fruit loss due to birds in Bangalore ranged from 30-36% in Arka Hans, Arka Shyam, Arka Kanchan and Bangalore Blue (Verghese, 1993) grape varieties. Birds also feed on packed berries. In home vineyards, bunches may be covered with muslin bags. The vineyards are also covered with nylon netting or electric fencing grid (1.5 volts). Bird scaring with traditional methods are also adopted (Jindal, 1990). The bat *Cynopterus sphinx* Vahl is a serious pest on grapes in Bangalore. Bat foraging was maximum at maturity of fruits and vines adjacent to open space had more bat damage. In Sikandarbad the short nosed fruit bat damage to grape bunches varied from 2.27 to 12.11 and degree of damage varied with the distance with the periphery of the vineyard (Srinivasalu and Srinivasalu, 2001). Erecting nylon netting around bower and covering the canopy gap on the bower with briar and twigs gave almost complete control of bat damage (Verghese, 1993; 1998) (Tables 1 and 2). Many of the pathogenic fungi developing on berries and leaves like *Cladosporium, Alternaria, Botryodiplodia etc.* originate from portions of vines receiving mechanical injury due to activities of vertebrates.

TABLE 1

Relationship between fruit maturity, size of fruit bunch and visibility of fruits in grapes (from Verghese, 1998)

Parameters	R	R^2
Fruit maturity	0.5502**	0.3002
Size of fruit bunch	-0.2840*	0.081
Visibility of fruits to the bats	-0.1465(NS)	-

*Significant at $p=0.05$; ** Significant at $p=0.01$; NS = Not significant

TABLE 2

Effect of zigzag netting on the damage caused by the bat, *C.sphinx* to grapes (from Verghese, 1998)

	Mean number of fruits damaged/vine	
	With zigzag netting	Without zigzag netting (control)
Pre-treatment	36.75	50.89
Post-treatment	58.37	164.34
Percentage increase in damage	37.04	69.03

3.3 Peach (*Prunus persica* Linn)

Roseringed parakeets, House crow and Jungle crow together caused 21.20% fruit loss in Punjab (Chahal, *et al*., 1973; Wagle, 1927; Singh *et al*., 1963; Toor and Sandhu, 1981; Mann, 1986). In Punjab, Flordasun Peach cultivar attracted flocks of House sparrow (*Passer domesticus* Jardine and Selby) during second week of January as birds and when new flush begin growing. As soon as the greenish-white flower buds appear, sparrows feed on flower buds to the extent that no flowers are produced. The average yield of Flordasun cultivar is 75 kg/tree. But the bird affected trees yield 0 to 2 kg only (Mann, 1986; Fryer, 1939). Field mice, *Mus booduga* Gray*,* Brown-spiny field mice, *Mus platythrix* Bennett, common house rat, *Rattus rattus* Linn and Indian Bush rat *Gulunder ellieti* constitute rodent pest species on plum, peach, apple and other orchards especially of nut fruits in Himachal Pradesh. Regular live trapping as part of non-toxic management approach was attempted in plum orchards in Himachal Pradesh. This method yielded 64.29, 62.50 and 50% reduction in population of *Bandicota bengalensis* (Toor, 1982). Provision of wild alternate foods in or at the vicinity of orchards lessened bird damage *e.g.* in Pear orchards (Grieg-Smith *et al*., 1983).

3.4 Citrus (*Citrus* spp.)

In hill region of Karnataka, comprising Shimoga, Hassan, Chikmagalur and Coorg districts, Oranges (*Citrus sinensis* L) are grown in coffee (*Coffea arabica* L and *Coffea robusta* L) estates (Ghosh, 1990). Shade trees planted or naturally present in estates, served as nesting and roosting sites for more than 90 bird species. Fruit losses due to various species of vertebrates on oranges is given in Fig. 1. Jungle crow was the dominant species feeding on oranges. An individual required 12±1.4 min (n=18) to empty the contents of a fruit. On an average, a crow destroyed 8 fruits/day and in 15 days, in the estate, crows destroyed over 2000 fruits. Goldenfronted Chloropsis (*Chloropsis cochinchinensis* Gmelin) siphoned out juice from the fruit by inserting beak into the rind while the fruit remained intact on the tree. Such fruits rot subsequently. Porcupines (*Hystrix indica* Kerr.) burrow at base and may dislodge young trees in severe cases of attack.

Depredated 19.6

Unaffected 80.4

| Monkey 1 | Jungle Cat 1.5 | Rat 1.9 | Jackal 1.7 | Birds 11.28 | Sqiorrel 2.8 |
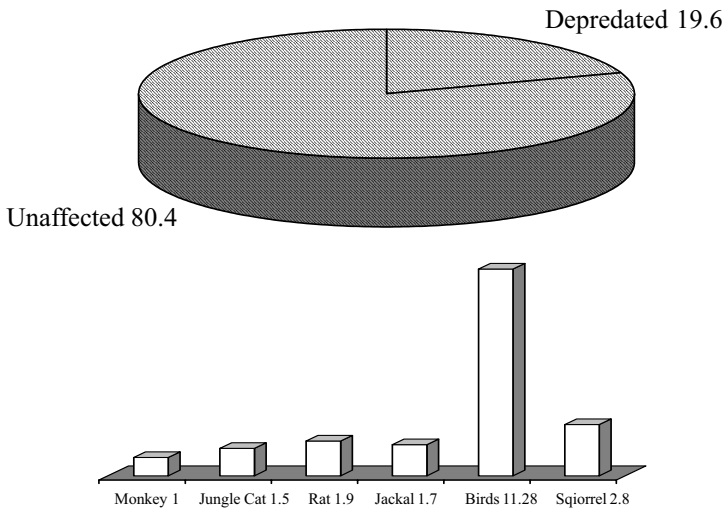
Fig. 1: Feeding losses in Oranges by birds in Mudigere

Monkeys (*Macaqua radiata* Linn), bison (*Bibos gaurus*), elephants (*Elephas maximus* Linn) and wildboar (*Sus scrofa*) caused damage to citrus orchards of all species in hill and coastal regions of Karnataka (Chakravarthy, 1993). While monkeys feed on fruits, others dislodge/detop/trample seedlings, break branches/twigs or peel off the bark while rubbing horns or body against the tree trunk. Subsequently the peeled off branches are prone to termites or barkborers attack. Orange fruit depredation by vertebrates was compared in 'watch/ward' orchard with no 'watch and ward' orchard from January to March 1993 at Mudigere, Chikmagalur. By watching 42% fruit losses could be prevented (Table 3) and there is further scope for saving fruits by effectively watching orchards and scaring away birds .

The fungus, *Sclerotinia libertiana* gets into wounds near the trunk on some of the main roots and rapidly produce large lesions and the entire tree gets a sickly appearance and there will be a reduction in the amount of fruits. The citrus ringsppot virus enters the tree through a vector or through mechanical injury resulting in a large number of irregular chlorotic patterns on mature leaves. Dieback caused by mycoplasma-like organisms and viruses, enter the plant through bruises caused by animals or due to nutritional disorders (Pathak,

TABLE 3
Effect of watch and ward on Orange fruit loss duet to vertebrate pests, Mudigere, 1992-93 (from Chakravarthy, 1993)

Dates	Fruit (Nos.) Loss/tree					
	Jungle crow		Other birds		Monkey	
	A	B	a	b	a	b
22.01.93	10	7	4	2	10	2
24.01.93	12	3	5	2	8	3
28.01.93	15	5	6	2	5	2
02.02.93	18	4	7	2	4	4
06.02.93	20	3	8	2	3	3
10.02.93	15	2	9	3	4	3
14.02.93	12	2	6	1	4	1
18.02.93	14	3	5	3	3	3
22.02.93	15	4	4	2	3	2
26.02.93	16	10	3	4	8	3
28.02.93	17	9	3	2	4	3
10.03.93	14	8	5	4	8	4
Total	178	60	65	30	64	34
Mean	14.8	5	0.54	2.5	5.33	2.83
% Reduction by watch and ward	66.29		53.84		46.88	
Binomial expansion(Z)	2.00		NS		NS	

A= without watch and ward   b = with watch and ward

1980). Apple crown gall is caused by the bacteria, *Agrobacterium rhizogenes* as a result of injury to roots or collar. Root rot and sap wood rot is caused by the fungus with white, mycelial mat on the bark. Injuries by rodents or physical injury caused during cultivation serve as points of entry for the fungi.

3.5 Guava (*Psidium guajava* L)

A review on the feeding habits of Roseringed parakeets (*P. krameri*) showed that the birds feed on seeds, berries, fruit, blossoms and nectar and are serious pests on guava. In Hyderabad, guava served as feed through out the year for the birds (Shivanarayan, 1982). While Singh

and Kumar (1982) studied the feeding habits. Verghese and Prasad (1985) and Prasad and Verghese (1985) studied foraging habits of the parakeet. Small green barbet (*M viridis*) and Jungle crow, *Corvus macrohynchos* damaged guava fruits in hill region of Karnataka, where birds on an average, incurred 14% unripe, 24% partially ripe and 33% ripened fruit loss (Chakravarthy, 1993). Birds gnaw even unripe fruits, wasting far more than they actually eat (Simwat and Sidhu,1973).The loss caused by birds to fruits was positively correlated with age of the fruit (Verghese and Tandon, 1993). Guava usually suffered 20% to 26% fruit losses due to birds in different parts of Karnataka (Chakravarthy, 1993). Redvented Bulbul, *Pycnonotus cafer* Linn and Red whiskered Bulbul, *Pycnonotus jocosus* Gould are minor pests. Indian flying fox, *Pteropus giganteus* B and squirrels (*Funambulus pennanti* Wroughtoni) are also major pests on guava.Monthly details of feeding by parakeet at Chethalli (Fig. 2).

In guava orchards, birds particularly parakeets caused so much loss that protection is warranted (Table 4). Shooting, trapping, scaring,

TABLE 4

Depredation by birds on Guava

Dates	Fruit (Nos.) damage/tree		
	Unripened	Partially ripened	Fully ripened
11.08.02	10.55	25.55	30.25
15.08.92	20.50	32.50	45.95
19.08.92	10.55	35.45	50.25
23.08.92	20.25	15.25	34.45
30.08.92	18.75	25.35	32.45
08.09.92	16.35	20.35	17.58
16.09.92	18.25	19.75	30.45
24.09.92	10.35	+35.25	43.75
02.10.92	08.35	12.35	20.35
10.10.92	06.25	15.45	22.15
Mean	14.02	23.75	32.86
CD 1%	Maturity=10.20	Dates=2.56	M x D = 0.8
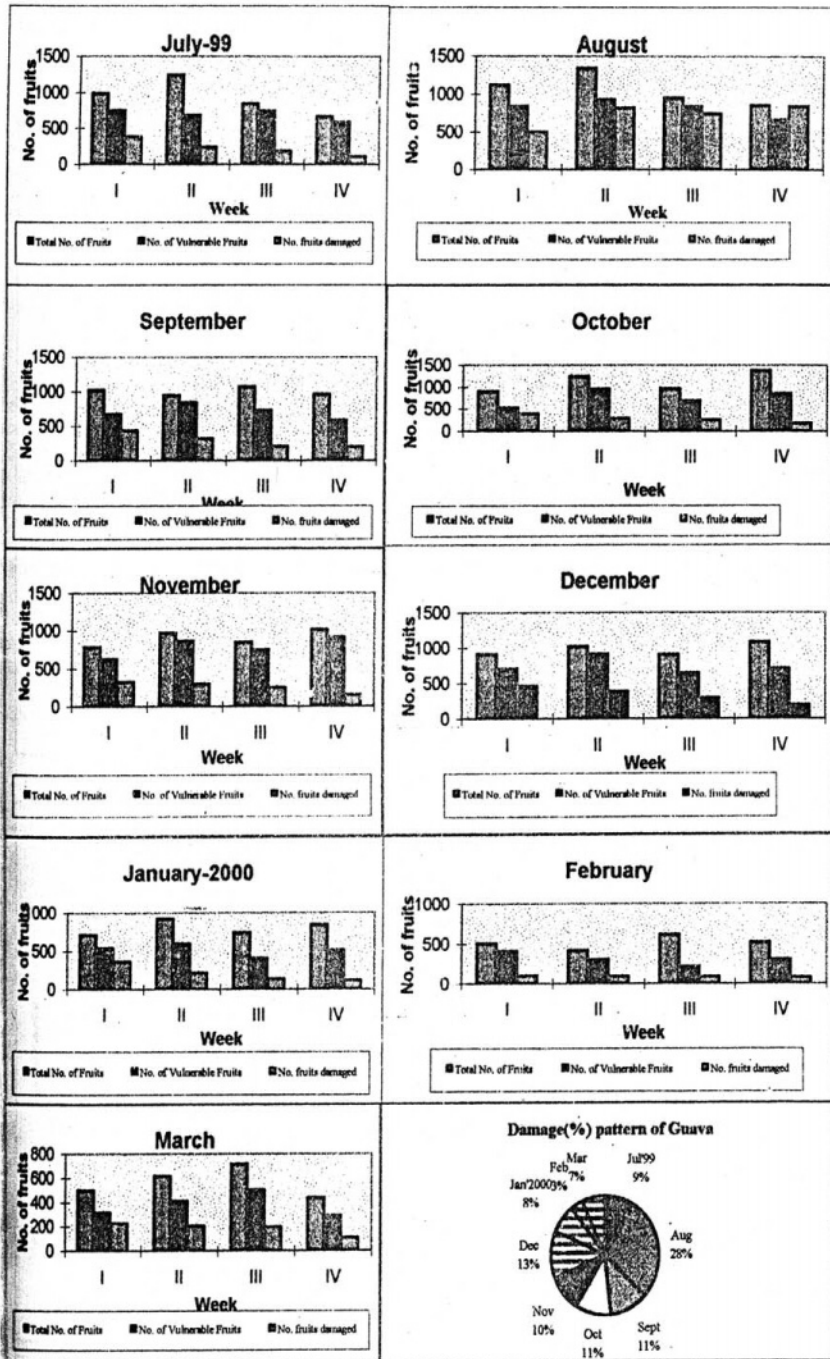
Fig. 2: Monthwise pattern of Parakeet damage on Guava at CHES-Chettalli.

destruction of bird roosts and nests and encouragement of natural enemies like owls help (Singh and Kumar, 1982). Covering vulnerable portions of canopy, during fruit-bearing period with thatch (locally available materials) saved 20% fruits in Mudigere, Chikmagalur (Chakravarthy, 1993). The stem canker incited on *Physalospora psidii* is caused due to lesions or cracks formed on the branches, which may be caused by vertebrates (Rajagopalan and Wilson, 1972). When fruits get injury, a number of pathogens like *Cylindrocarpon* sp. infect fruits (Jamaluddin, 1976).

3.6   Sapota (*Achras zapota* L)

Jungle crow (C. m*acrorhynchos*) and House crow (C.s*plendens*) caused a loss of 12% fruits under Bangalore conditions. The crows are major pests while Redvented Bulbul is a minor pest. In Mudigere, Chikmagalur, Crows (*Corvus* spp), monkey (*M.radiata*), Squirrel (*F.palmarum*) and bats (*P.edwardsii*) caused a loss of 18% fruits in a season. In Chethalli, Coorg, large fruit eating bat, *P.edwardsii* and short nosed fruit bat, *Cynopterus sphinx* caused on an average, a fall of 3 fruits/tree as bats were also observed carrying fruits to their perch. These species also damaged tender coconuts, mango and guava (Chakravarthy, 2000) in maidan areas of Karnataka. In Bangalore, rodents damaging sapota included *Bandicota bengalensis* and *Tatera indica* Jerdon. Population of the rodents was high during October to December coinciding with fruit bearing and harvesting stages. *B.bengalensis* was the dominant species at all stages of crop growth. Sapota like orchards of mango, citrus, guava, litchi *etc.* are not free from raids by elephants (Loyttyniemi and Mikkola, 1990). Bisons too caused either bark splitting or debranching . Sapota fruits were damaged to the maximum during january (Fig. 3a,b,c).
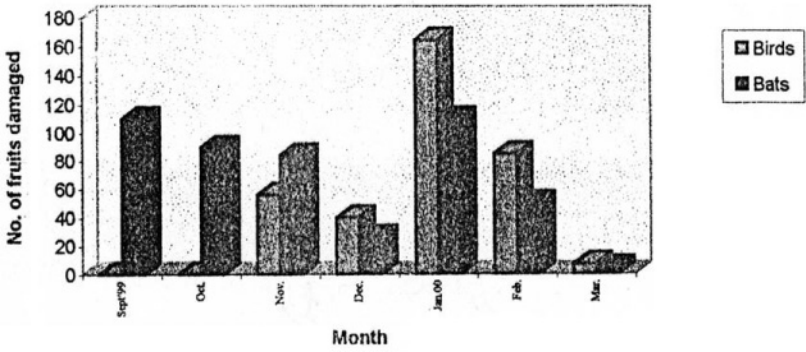
Fig. 3a : Sapota fruits damaged by birds and bats during different months at CHES
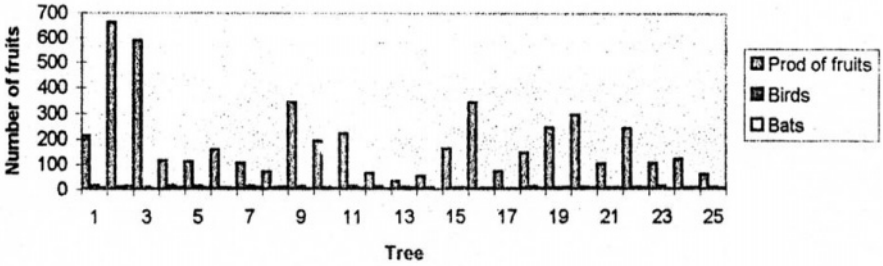
Fig. 3b : Number of fruits produced and damage by birds and bats against individual free (December, 31 days)
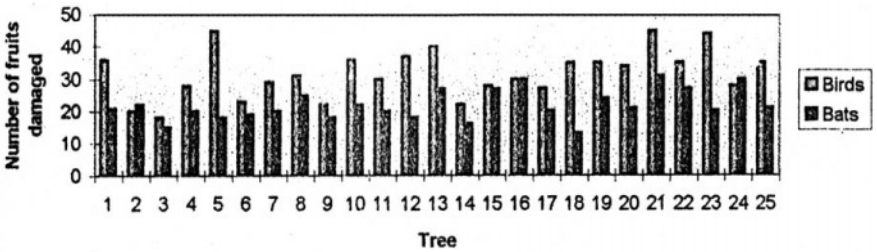
Fig. 3c : Tree wise damage pattern by vertebrate past species against GBH (90 days of damage)

3.7 Banana (*Musa* spp.)

Banana(*Musa paradisiaca* L.) preferentially damaged by elephants, bison and wildboar. At Chethalli, Coorg, germplasm maintenance of banana had to be abandoned due to raids by bisons and elephants. Elephants split the pseudostem vertically to feed on pith. Despite one ha of Neypoovan banana orchard being lighted by electric bulbs, bonfire and patrolled by men daily, elephants continued damaging banana at Chethalli (Chakravarthy, 1993).

At Ujire, Belthangady taluk, South Kanara, three varieties namely, Mysore bale, Nanjangud and Nendran were planted. The banana fruits were depredated by monkeys (*M.radiata*), barbet (*M.viridis*) and bats (*P.edwardsii*). The barbets damaged ripening fruits. The bird worked on the bunch to get beakful lumps of pulp. A bird damaged 1 to 2 fruits/bunch and continued to forage in search of next ripening bunch. It was found that by retaining damaged fruits on the plant, bird damage to banana was lessened. This was because birds revisited the damaged fruits to feed on the sweetened fruit portions. Interestingly, birds attended on Nendran variety when the fruits were still green. Damage on fruits of other varieties began when the fruits showed yellow color. The preference by birds was in the order: Nendran > Nanjangud > Mysore. The Mysore variety fruits were the least preferred because it secreted a mucilage ooze which rendered the fruits distasteful. Isolated banana orchards were prone to heavier vertebrate pests damage.

Leaf and fruit spots is caused by *Helminthosporium torulosum* (Mitra, 1930). whose pathogenicity has been established on leaves and fruits and found that injury and high humidity and temperature favour spore liberation. Improved sanitation, destruction of infected plant parts, good drainage and repeated applications of captan or Dithane Z-78 @ 2 g/litre and prevent the plants from the disease. Another wound fungal pathogen *Botryodiplodia theobromae* causes fruit rot which results in rotting of pulp (Bhargava *et al.,* 1965).

3.8 Mango (*Mangifera indica* Linn)

Roseringed parakeetes feed both on immature and mature mango fruits. In Chethalli, Coorg, mango is damaged by bisons and elephants,

breaking branches, splitting the bark or uprooting young (<5 years old) trees was common.

A number of frugivorous birds like Hill myna, *Gracula religiosa* Cuvier; Indian white-eye, *Zosterops palpebrosa* Temminck; Barbets, *Megalaima* spp. Goldenbaked woodpecker, *Dinopium benghalense* Malherve*; Indian lorikeet, *Loriculus vernalis* Sparrman; Blossomheaded parakeet, *Psittacula cyanocephala* Linn; Large Indian parakeet, *Psittacula eupatria* Hodgson; Roseringed parakeet, *Psittacula krameri*; bats like *Cynopterus sphix, Pteropus* sps., mangoose, *Herpestes edwardsii* L, rodents like, *Ratufa indica* W, species of *Funambulus, Rattus rattus*; monkeys like *Macaqua radiata*; langurs like *Presbytis entellus* Linn *etc*. have been recorded feeding on mango frutis. These animals leave partially injured fruits, branches or roots in orchards, where bacterium like *Xanthomonas campestris* is found throughout the year and enters through injuries causing bacterial spot. Rainfall and high wind velocity are favourable for spread of the disease (Singh, 1998).

Sooty mould fungus, *Capnodium mangiferae* enter into tissues not only on the honey dew exerted by various insects, but also due to injury by other animals. This fungus interferes with the photosynthetic activity of the plant resulting in stunted growth and poor fruit setting. Pink disease is a fungal disease of minor importance caused by *Pellicularia salmonicodor*, which appears as a pinkish powdery coating on infected twigs, stems and branches, which often spreads and girdles the affected parts. Pathogen also invades the tissues and interfere with the transport of nutrients. As a result, the branches wither and dry-up, while the leaves are shed (Pathak, 1980).

3.9   Pineapple (*Annas comosus* L)

On an average, pineapple (*Annas comosus*) fruit losses due to vertebrate pests (jungle crow, squirrel and rat) amounted to 22% and 12%, respectively in coastal and hill regions of Karnataka. In a field trial, 70% of fruits uncovered were destroyed by vertebrate pests compared to no damage on fruits covered with either leaf or dry thatch (Chakravarthy, 1993). Fruit losses due to vertebrate pests were reduced by effective watch, timely harvests, covering fruits with thatch

and by driving away the animals (Nagarajan, 1994). Mongoose, *Herpestes edwardsii* posed the major threat in some areas.

3.10   Ber (*Zizyphus* sps.)

Both immature and mature fruits are attacked by Roseringed parakeets in Punjab. In North-West desert of Rajasthan, gerbil, squirrels and rats are the major pests. As cultivation of ber is not on commercial lines in most parts of the country, crop protection measures are seldom adopted.

3.11   Pomegranate (*Punica granatum* L)

Squirrels of species of *Funambulus* are the principal pests feeding on pomegranate fruits throughout India. The damage starts when the fruits are ripe (Sandhu and Dhindsa, 1995). The partially damaged fruits are left behind and the animals search fresh fruits for feeding. The fruit loss is cent per cent during summer (February to May) when alternate food sources are scarce. Species of *Bandicota* and *Rattus* work on such damaged fruits during night and empty the contents. Seeds are extracted from damaged fruits by birds like Redvented Bulbul (Patel, 1993).

Snap trapping, sticky bands, covering fruits with polybags, cloth-bags, bags laced with waste oils, etc. were the methods adopted to protect the fruits under Bangalore conditions in small orchards (1 ha) and backyards of houses. Ripening fruits of pomegranate and custard apple laced with oils of neem (*Azadirachta indica* L) and castor (*Ricinus communis* E) protected the fruits from vertebrate depredations for a longer period (Chakravarthy, 1993).

3.12   Litchi (*Litchi chinensis* Linn)

Litchi fruits are attractive to crows, bulbuls, sparrows and bats, *P.edwardsii* in South India and *P.giganteus* in North India. While birds feed on fruits, the bats in addition, carry the fruits away (Chakravarthy, 1993). Leaf spots incited by *Pestalotia pauciseta* occur in Lichi orchards as a result of injury to leaves. The diseases leaves serve as a source of infection to the injured ripe or unripe fruits .

3.13   Jamun (*Syzygium cumini* S)

Roseringed parakeets feed on both immature and mature fruits (Toor, 1982; Singh, 1982; Singh and Kumar, 1982). Under Bangalore conditions small green barbet, coppersmith (*Megalaima haemacephala* Lathm) and mynas of *Acridotheres* spp. were observed feeding on fruits (Chakravarthy, 1993; Lal, 1959, Mehrotra and Bhatnagar, 1979).

3.14   Papaya (*Carica papaya* L)

Ripe fruits of Papaya are damaged by birds, squirrels, monkeys and elephants. Usually development of yellow colour marks the beginning of bird damage. Two, green barbets *M.viridis* consumed on an average, pulp of a medium sized papaya in 12 min (n=8) in Belthangady, South Kanara. In Chethalli, Coorg an acre of 'honeydew' was raided by elephants. A troupe of five elephants damaged 8% of five months old plants. The wildboar squeezes the stem tissues and detops the plant. At times the wildboar was found browsing on foliage of young papaya plants (Chakravarthy, 1993). Covering fruits with thatch gunny bag or a festoon of thorny sticks or nylon nets and scaring, facilitated management of vertebrate pests.

3.15   Cocoa (*Theobroma cacao* S)

In South India, cocoa is cultivated with areca, coconut, coffee and cardamom or as a pure crop in small holdings upto 2 acres. Jungle cat *Felis chaus*, squirrel, *F.palmarun*, rat *R.rattus*, monkey *M.radiata*, jackal *Capra aureus*, bison. *Bibos gaurus,* stag *C canis* and birds constitute vertebrate pests complex. Fruit setting starts from July and pods continue growing till December in hill region of Karnataka. Squirrel damage commenced at the beginning of the fruiting season July-August (Fig. 4), Jungle cat during pod ripening stage (October-November) and rat damage, throughout the year (Table 5). Monkey, jackal and birds were found damaging pods during ripening stage. Jungle cat is the most important pest (Table 6) incurring over 20% losses in pod yields in hill region of Karnataka and loss due to all
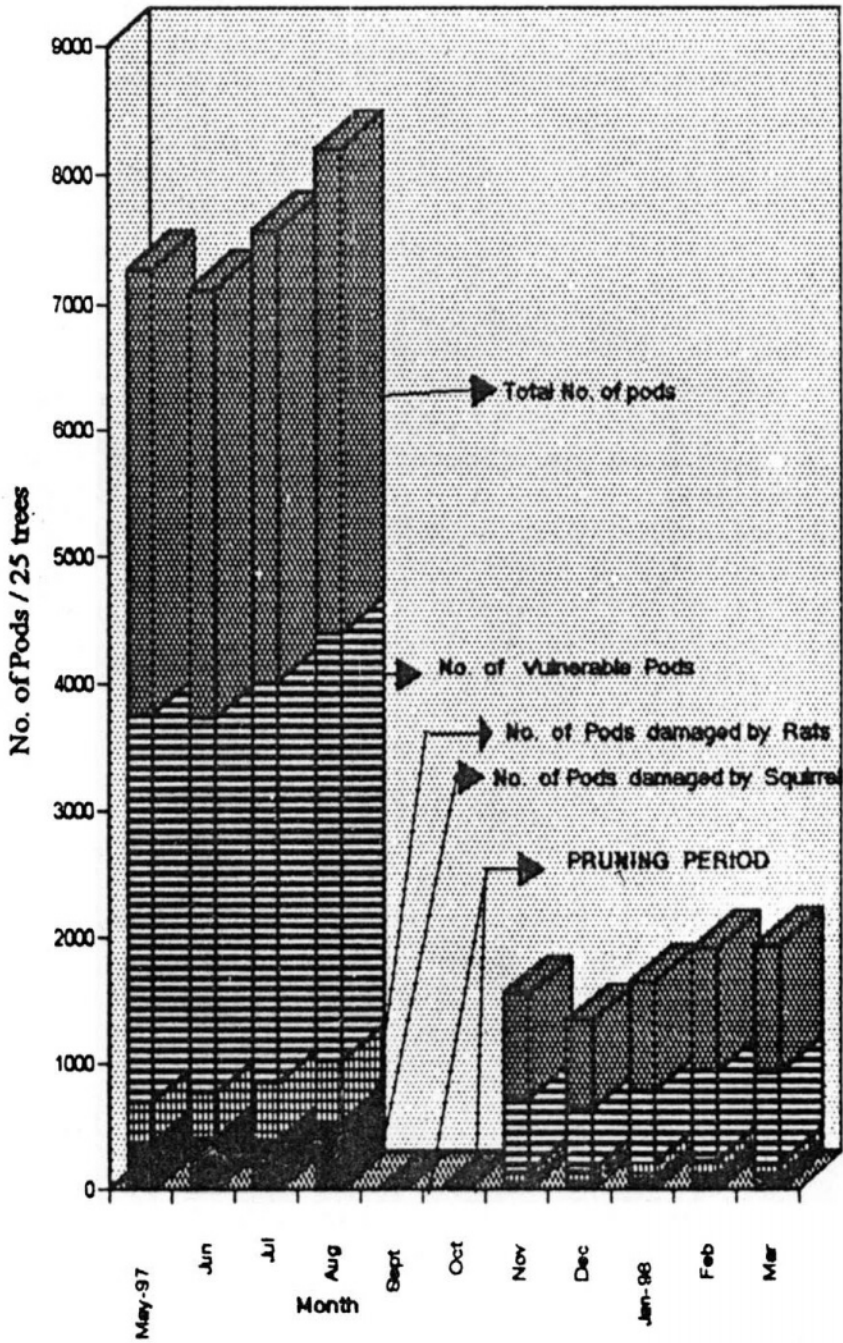
Fig. 4 : Vertebrate pest damage to cocoa pods at CPCPRI-Vittal, Dakshir coastal Karnataka.

TABLE 5

Vertebrates damage to cocoa pods (in hilly and coastal
zones of Karnataka during 1997-98)

Month	Pods damaged by squirrel	Pods damaged by Rat	No. of vulnerable pods	Total No. of pods
May –97	358	331	3067	3509
June	397	360	2967	3388
July	389	450	3170	3561
Aug	529	486	3375	3807
Sept	0	0	0	0
Oct	0	0	0	0
Nov	51	80	579	842
Dec	56	79	493	708
Jan-98	81	118	575	872
Feb	114	113	699	980
Mar	94	101	724	1012

TABLE 6

Vertebrates cocoa pods in hill region of Karnataka
(from Thyagaraj *et al.,* 1966)

Location	Jungle Cat	Monkey	Squirrel	Rat	Jackal	Birds
Sringeri	29	1	01	01	0	1
Koppa	24	0	02	01	0	0
Kalasa	07	1	13	07	1	1
T.halli	09	0	19	13	1	1
Sagar	18	0	15	04	1	5
Soraba	14	1	11	17	12	2
Sirsi	43	2	27	19	19	1
Total	144	5	88	72	34	11
Mean	20.5	0.71	12.57	10.28	4.85	1.57

*Actual pods damaged ; Mean of two seasons data

vertebrates exceeded 35% yield losses due to damage caused by different types of vertebrates in hill regions of Karnataka has been assessed (Chakravarthy, 1993 and Thyagaraj *et al.*, 1996). Cocoa plantations near forest tracts are attacked by bison which detops the plant and often replanting is required in the plantations. Cocoa adjacent to forest suffers the heaviest damage largely because of its seasonal cropping pattern as cocoa forms only a part of the diet of mammalian pest species. During 1992 and 1993 a baiting trial with carbofuran 3% G and warfarin 0.05% simultaneously with wooden snap trapping (5 to 6 traps/acre) was undertaken at three localities (Table 7) and local banana fruits 'Puttabale' served as poison carrier. The number of healthy pods/plant increased significantly in the treated, compared to untreated plots. In northern Karnataka, wet jaggery and dry sea fish are used as poison carriers.

TABLE 7

Effect of poison baiting on vertebrates feeding on cocoa pods
(from Thyagaraj *et. al.,* 1996)

Location	Treated plots Healthy pods per plant before treatment	Control plots Healthy pods per plant after treatment	Healthy pods per plant at beginning of season	Healthy pods per plant at the end of the season
Nemmar	06	40	11	5
Huguluvalli	09	28	13	3
Kalpatharu	11	33	14	2
Total	26	101	38	10
Mean	8.68	33.66	12.766	3.33

*0.05% Carbufuron 3% g; 0.05% Warfarin; **200 No.of plants/location; Mean of two seasons

3.16   Coffee (*Coffee arabica* L and *Coffea robusta* L)

Coffee (*Coffea arabica* and *Coffea robusta*) is the crop that holds the maximum number of vertebrates that interact actively with the elements of the ecosystem. The main reason is that coffee ecosystem most closely represents the natural forests and represent the minimal

modifiers of habitats for wild animals and plays an important role in vertebrate pest management in the region. Among 91 bird species recorded throughout the year in coffee estates of hill region of Karnataka, only Small green barbet and Red whiskered bulbul (*Pycnonotus jocosus*) attained economic status (Fig. 5). These birds fed only on ripened coffee berries. Birds punctured the pericarp and siphoned in the sweet contents and dropped the husk and seeds on the ground. Birds in general, are potential predators of arthropods and pollinators than depredators. Monkeys, bison, wildboar, rats, squirrel and elephants caused negligible damage (Chakravarthy, 1993; Bhat *et al.*, 1995), de-branched coffee bushes and affected the crop for the next year. So the loss caused by monkeys is economically important. Monkeys also feed on sweet, succulent and palatable stem tissue of young plants. Timely scaring using trained dogs effectively prevented the loss. At this juncture, management practices against vertebrates are not desired (Loyttyniemi and Mikkola, 1990).

The normal resistance of healthy coffee, green berries is reduced when the tree is under stress or wounded because fungi can infect immature fruits resulting in light or empty beans. Thus, reducing both quality and quantity of the harvested crop. Some yeast – like

Relative Damage

Unattected 80.4

Depredated 19.6

Monkey 1
Jungle Cat 1.5
Rat 1.9
Jacket 1.7
Birds 11.28
Squirrel 2.8

Percent Damage
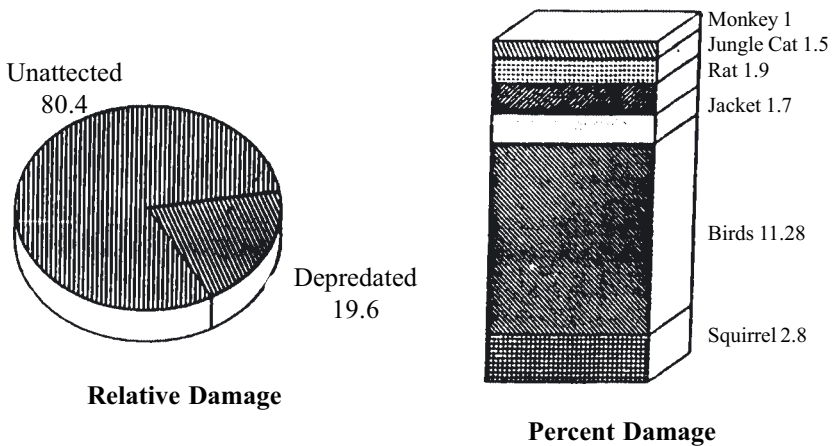
Fig. 5 : Vertebrates on coffee in Mudigere : species composition and relative damage

fungi (*Nematospora* spp.) infect berries and cause blight. Beans can produce off flavors when the coffee is wet processed due to decay web blight of branches (*Corticium* sp.) causes pink disease in coffee bush-branches in humid areas, when branches bear buises or cuts due to animals interactions.

3.17 Coconut (*Coccus nucifera* L)

The coconut palm is depredated by vertebrates right form the time of sowing to maturity . Growers are currently using indigenous devices and tools to protect the palms from vertebrates. However, the efficacy of these traditional methods is too low (Bhat *et al*., 1990; 1995). Damage to coconut by monkey, rat and wildpig at five localities over eight months in coastal region of Karnataka showed that vertebrates caused economic losses in coconut. Monkeys damaged maximum number of nuts (150 to 200/15 days in March 1997), rodents mainly *B.bengalensis* damaged 15 to 20 nuts/15 days and wildpig the least, 0 to 4 nuts/15 days at Ennakala, South Kanara, Karnataka (Table 8). ANOVA revealed significant differences in nut damage caused by three species of vertebrates. Obviously wildpigs targeted only the fallen

TABLE 8

Damage to coconut by three vertebrate species (From IVPM in hilly and coastal zones of Karnataka during 1998-1999)

Month	Total nuts damaged			
	Monkey	Rat	Wildboar	Total
Sept '96	175	18	13	206
Oct	200	04	04	208
Nov	052	00	04	056
Dec	075	00	00	075
Jan '97	113	00	00	113
Feb	082	11	02	095
Mar	151	00	00	151
Apr	051	22	03	076

nuts. But rot incited by *Phytophthora palmivora* can be disseminated by wind and insects and vertebrates attack can predispose the palms to the disease. Similarly vertebrates can play a role in causing stem bleeding, root diseases (Pathak, 1980).

Based on the score, (function of palm height) bison damage to coconut palms was graded. The bison damage in the field commenced from July. It was severe during August to November and the damage was related to biomass of grasses in the field. Grasses probably stimulated the animals to damage the crop. The number of animals increased at the rate of one bison/18 months. Porcupine (*Hystrix indica*) damage to East coast tall coconut at Kidu farm (Fig. 6), South Kanara was quantified based on the height from base to which the palm was debarked. The animals chipout bark pieces and then burrow into the soil, rendering the stem hollow.
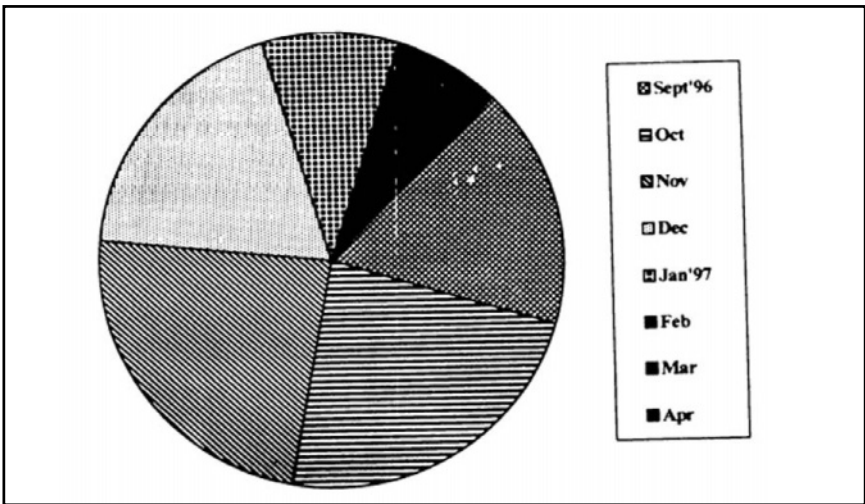
Fig. 6 : Porcupine damage to Andaman ordinary (coconut), at Kidu, Subra.

Upto July, no damage was observed and during this period grasses were cut and basins of palms remained open. This deterred the rodents. Presence of grasses promoted porcupine damage to palms (Table 9). This supposition gains support from the fact that during the same period palms in the forest tract were damaged and base of these palms were covered with grasses.

Coconut palms are also raided by elephants. Observations during the last decade revealed that in Coorg, Karnataka, maximum damage

TABLE 9

Efficacy of cocoa palms against porcupine damage in hilly and
coastal zones of Karnataka, during 1998-1999

Sl.No.	Treatment	No.of days for which the palm remained protected
1.	Lacing coal tar	60
2.	Lacing Japan black	95
3.	Lacing waste lubricant oil	45
4.	Covering base with bamboo thatch	210
5.	Covering base with metallic mesh	250
6.	Application of 10 G Thimet	30
7.	Application of Racumin	40

(8%) occurred during July. The damage embraced trampling, browsing,
uprooting and detopping of coconut palms.

Trunk banding (metal) (Table 10), burrow baiting against rodents,
clean basin cultivation, crown cleaning, lacing base of grown-up tree
trunks with waste oils or covering base of seedlings with thorny
bamboo or a mat of dry sticks against porcupine and timely harvests
and pick-up of fallen nuts have proven effective. It is important for
the manager to distinguish damage to the major vertebrate pest species
like rat before adopting practices like lacing tar, oils, *etc.* Fallen nuts
in coastal Karnataka are mainly damaged by wildpig .

TABLE 10

Pattern of damage of sapota by vertebrate pests at 3 different
stages of the crop (in hilly and coastal zones of Karnataka
during 1998-1999)

Plot	Month	% damage by		
		Monkey	Bat	Wildlboar
Ennakala	Dec '96	100	00	00
Harpadi	Nov	100	00	00
	Dec '96	100	00	00
Eramadaka	Nov	100	00	00
	Dec '96	100	00	00
Charmadi				
1) Abdulla plot	Sep '96	78.80	21.20	00
	Nov	61.50	24.80	13
2) Anantha Rao's plot	Sep '96	61.80	38.20	00
	Oct	58.90	41.10	00
	Nov	84.00	16.00	00
	Dec	73.80	26.20	00
Average damage (%)		83.52	15.23	1.25

3.18   Arecanut (*Areca catechu* L)

Arecanut in coastal region of Karnataka starts flowering form March-April, ripening of nuts from September and first harvest occurs in October-November. The harvest ends by March. Damage by vertebrates begin from October. *i.e.* at ripening stage. Monkeys, rats, wildpigs and bats damaged nuts. Monkey damage on nuts was identified by the irregular peeling off fruit epicarp, partially or completely. The unriped fruits were plucked off and dropped. Such nuts carried stalks and tooth marks. Bat damage was restricted to ripened or over-ripened leaving the fruit surface damaged and exposed for pathogenic infection. Wildpigs fed only on the fallen ripened nuts. It chewed the whole nut, leaving the crushed nuts. Comparative pattern of damage to areca by vertebrates in coastal region of Karnataka is documented, (Bhat, *et al.,* 1990). Nuts damaged by monkeys are collected and sold by farmers as second quality nuts at 20% reduced rates than healthy nuts.

Growers protected the crop by gun patrolling Ibex fencing, cracker bursting, scaring and by lighting and repellant odours like waste tyre burning, bonfire, *etc.* Damage to areca seedlings from wildpig and rats could be avoided by using locally available porcelain pipes. Monkey damage could be reduced by disfavouring the habitat. Even roosting of Roseringed parakeets caused damage to foliage. By regular patrolling, clean cultivation and burrow baiting with zinc phosphide (0.005% rodafarin) rodent damage could be reduced. A rigorous and continuous guarding the plantations, proved costly and impracticable. Ibex fencing, solar fence, timely harvests, selective debranching of shade trees at edges and clean cultivation ameliorated the problems due to vertebrates to some extent (Chakravarthy, 1993).

3.19   Cashew (*Anacardium occidentale* L)

Eight species of birds depredate cashew apples. Deer, *Cervus axis*, squirrel, monkey, wildlboar and porcupines (*Hystrix indica*) damaged cashew in hill region of Karnataka. Seedlings are damaged by rats, porcupine and wildlboar. The total loss due to vertebrate pests in cashew at Mudigere, Chikmagalure was 17% of apple and 21% of

nut during 1991-92. During April-May 1991, a troupe of eight monkeys, on an average (n=104) caused 24% nut losses (Chakravarthy, 1993). Rai (1984) recorded house crow, roseringed parakeet, fruit bats, pangolin (*Maris temmeneki* T), porcupine, house rat *R.rattus*, squirrel, *F.palmarum*, Jackal (*C aureus*), marmoset (*Callituris jacchus* W) and monkey *M.radiata* depredating cashew apples and nuts in coastal region of Karnataka.

Bats and birds usually carry a large number of nuts that can be collected under the trees on which they roost and hence are responsible for losses in yields. Nuts littered on ground under roosting trees are collected and auctioned. Porcupines and bandicoots damaged seedlings by burrowing at the base and damaging underground parts and boll region. During the fruiting season rodents damaged nuts and apples. Jackals fed on apples alone and left the nuts. Monkeys are fond of ripe cashew apples and sometimes damaged nuts. Raids of elephants and bisons caused debranching and bark peeling. Crop protection measures against vertebrates are not adopted (Chakravarthy, 1993).

3.20   Nut fruits

Nut fruits like walnuts, almonds, apricots, Pecan nuts, *etc. a*re damaged by squirrel of *Funambulus* sp., birds like mynas, bulbuls, crows, chloropsis, monkeys and rats. Crown galls caused by *Agrobacterium tumefaciens* enter through wounds caused mechanically or by vertebrates. Soft, sprongy galls are formed on roots or trunk and trees become stunted . Nut fruit trees are also infected by bacterial gummosis or bacterial shoot blight caused by *Pseudomonas syringae* where by gumming lesions arise on bark or outer sap wood and fruit. Under severe conditions, terminal die back of shoot occurs.

Birds carry the chestnut blight fungus, *Endothia parasitica* which spreads locally by air borne ascospores discharged from perithecia in wet weather. But outbreaks in new areas are often caused by insectivorous birds. Old cankers of chestnut blight are infected with boring insects for which birds especially woodpeckers search and then become contaminated with pyncospores. Leach (1940) pointed out that woodpeckers feed on tree cambium as well as on insects and so could easily infect healthy trees.

4   Phanerogamic plant parasites on orchard crops

Phanerogamic parasites on orchard crops are mainly of two types *i.e.* stem parasites and root parasites Vertebrates are implicated in the spread of phanerogamic plant parasites in orchards and gardens.

4.1. Stem Parasites

a)   Holoparasites (entirely dependent) – *Cuscuta* sp.

The parasitic angiosperms, a diverse group, lead a hemiparasitic (partial dependence on host) or holoparasitic (total dependence) mode of life. All holoparasites and some of the hemiparansites have replaced their normal root system by haustoria, while many hemiparasitic plants possess both a root system and haustoria. The haustoria effectively replace the root system, and their presence could simply and conviniently be interpreted as an adaptation to suit their parasitic mode of life. Their development provides a most effective and intimate region of contact between the host and the parasite in order to establish pathway for the nutrition of the later (Bhandari and Mukerji 1993; Fineran, 1987).

b)   Hemiparasites / Semi-parasites (Partially dependent) – *Loranthus, Dendrophthoe*

Dendrophthoe is a common parasite of fruit and roadside trees. Its sanskrit name is "*Vrikshabhaksha*" meaning eater of trees. In India, mango trees are the worst sufferers from this parasite. In northern India 60-90% of the old, desi type mango trees and a large number of other trees are parasitized (Bhandari and Mukerji, 1993).
    Dendrophthoe fulcata, is a strongly branched and glabrous shrub. The stem is thick, erect or flattened at the nodes and appears to arise in clusters at the point of attack. This cluster forms a dense and bushy growth which can easily be spotted on the trees. The place at which the host is attacked and where the haustorium penetrates, often swells to form tumors which vary in size according to age of the parasite. Sometimes, the parasite, instead of confining its attack to one point, produces a creeping branch which grows closely along the host stem

and forms haustoria at intervals. The fruit is fleshy and contains a solitary seed. It is sweet and eaten by birds, cattle and other animals (Singh, 1998).

The parasite is spread by dispersal of its seed mostly through birds and to some extent by other animals. When the seeds get deposited on other trees at the junction of branches with the trunk, they germinate and give rise to haustoria, establishing the parasite. Droppings of birds containing seeds also help in dissemination of the parasite (Singh, 1998).

In early stages of the attack, the damage to the tree may not be appreciable but later the parasite increases in vigor and the effects become apparent. Beyond the point of attack fresh growth of the host shoot is stunted. The damage done by the parasite is most marked in the production of new growth by the host. The quality and yield of fruits is considerably lowered. Leaves may be reduced in size which is usually well marked in mango. The effect of the attack also depends upon the vigor of the host tree. A large tree, if mildly attacked, will not show any effect. The same parasite is noted on sapota and jamun trees in Karnataka.

The commonly known method of control of the parasite is to top off the infected branches. It is important that branches should be cut sufficiently low, so that all vestiges of the haustorial system of the parasite are eradicated. In early stages of the growth of the parasite it can be easily detached from the host without damaging the latter. If the tumor is on one side of the branch then the wood just below the tumor may be sawed off. Injection of copper sulphate and 2, 4-D into affected branches has been found effective on many hosts. A spray of diesel oil emulsion in soap water is also effective in eradicating the parasite from mango trees (Singh, 1998).

4.2. Root Parasites

The root parasites belong to families Santalaceae, Lennoaceae, Orobanchaceae and Rafflesiaceae. The germination of *Orobanche* sps. and *Striga* sps. seeds is host dependent. It is possible to trigger the process of seed germintion by the application of extracts from host plants, indicating the presence of some factor/s which stimulates

germination of seed (Al-Menoufi *et al*, 1987) *Orobanche* and *Striga* require associated hosts (Sahai and Shivanna, 1982).

5. Integrated Vertebrate Management

For the management of vertebrate in orchards, a compatible combination of mechanical, cultural, biological and nature friendly chemicals were integrated and tested in replicated plots in hill regions of Karnataka. Results of selected experiments are summarised in following paragraphs.

The vine yards are covered with nylon netting or electric fencing grid (1-5 volts) covering the canopy gap on the bower with briar and twigs. This gave almost complete control of bat damage. Provision of alternate foods at the vicinity of vine yards and timely harvests also reduced fruit damage due to vertebrates in and around Bangalore (Verghese, 1993; 1998). Grape wines grown under net mesh supported by vertical poles form continuous complex of leaves and branches in which bunches are hidden. Grape growers use a variety of methods like netting and fire crackers (Srivasulu and Srinivasulu, 2001).

A systematic baiting trial undertaken at Kuruvalli and Hugluvalli at Thirthahalli, Shimoga and at Addegadde, Sringeri, Chikmagalaur in 6 ha of cocoa plantation from 1991 to 1993 and observations in different taluks in Dakshina Kannada district from 1985 to 1993 revealed that cocoa plantations were effectively protected form jungle cat, rodents, jackal, monkey and stag damage. Carbofuran 3% G baiting in local banana fruits or wet jaggery, intercropping with areca or coffee, timely harvest, clean cultivation, snap trapping at peak pod production period (August/September and December/January) effectively protected the crop (Thyagaraj *et al.,* 1996).

A three years trial in arecanut and coconut gardens at Dharmasthala revealed that clean basin cultivation, crown cleaning, trunk banding, burrow baiting, Ibex fencing with atleast one metre of open space on either sides and covering base of seedlings (upto 2 to 3 years) with tightly packed cover of thorny bamboo sticks or porcelain pipes wherever the coconut and arecanut palms were regularly prone to rodents (rats, bandicoots and porcupine) damage saved 46% nuts

compared to 'control' against rodents, monkey and wildboar depredations. These practices to some extent afforded protection against rodents, monkey and wildboard depredations, and also against raids by bison and elephant.

In oilpalm, *Elaeis guineensis* Jacq. covering ripening bunches with gunny bags or thatch of farm wastes or paddy straw, timely harvests and scaring away vertebrates by odours, acoustic or watch and ward saved 62% fruits from birds (crows, mynas and parakeets) rodents (rats and bandicoots) and wildlboar compared to control. These practices showed consistent results under heavy animal depredations.

The above instances of crop protection against vertebrates demonstrated that it is possible to protect fruit crops by cultural and crop husbandry practices that also helped in harvesting sustainable crop yields. The practices were cheap, practicable ecofriendly and harmless to non targeted species including humans.

6. Constraints

It is clear that barring few species that are host-specific or have restricted geographical distribution, the same vertebrate species is implicated in causing damage to more than one fruit crop. This is because of the preference and adaptiveness of the species to forage in several cultivated ecosystems. Determination of economic status and ecological roles of vertebrates in their natural habitats and cultivated ecosystems is important (Srihari and Chakravarthy, 1998). For instance, adult House sparrow feeds on fruits and nuts but feeds its young ones with insects, some of which injure orchard crops. Similarly rats, squirrels and porcupines which variously damage a variety of fruit crops, are important regulators of surface and sub-surface vegetation, soil fertility and soil fauna. Perhaps with the exception of Roseringed parakeets and few rodents of species of *Rattus* and *Mus* and bandicoots of *Bandicota* all other animals perform diversified roles in their habits and cultivated ecosystems (Hussain and Bhalla, 1937). Determination of economic status of vertebrates is therefore

difficult and complicated. But studies with multidisciplinary approach by teams of workers in this direction are urgently required. Destruction of plant parts damaged by vertebrates, sanitation, application of fungicides or appropriate chemicals on the wounded or injured plant parts, fencing, provision of alternate foods of animals in orchards *etc*. can minimise vertebrate damage to fruit crops as well as the chances of fresh infection. Little work has been done on the passage of pathogens through the intestines of animals other than insects. More critical experiments are needed before significance of vertebrates as vectors of pathogenic organisms can be assessed. Animals provide wounds for the entry of *Pseudomonas* into plant parts.

The traditional approaches to crop protection from vertebrates depredations have been reported by many workers (Gee, 1951, Fitzwater and Prakash, 1973, Shuyler, 1972, Sinclair, 1894). There is a need to scientifically validate local methods and improve their effectiveness and flexibility to varying conditions of orchard systems, if possible.

In many places in India entomologists are looking after vertebrate pests problems in the zones. There is a lack, in general, of trained personnel in vertebrate pests. There should also be a network among vertebrate researchers in universities and institutes with ecologists and vertebrate pest managers.

No programme of pest management in horticultural systems can be effective unless it is based on knowledge of the animal's natural history (Strendale 1894, Taber *et al*., 1967, Wagle, 1927). Studies of habitat preferences should be combined with records of seasonal population changes so as to provide basic information for pest management (Prakash *et al.,* 1971, Mohana Rao, 1992). Telemetry is by far an accurate means of monitoring activities of vertebrate pests, two years data on population fluctuation should be gathered. Hone (1994) described and critically reviewed literature on a range of analysis used in vertebrate pest research and management. Statistical, economic and modeling analyses are described with spectrum of damage by vertebrate pests and the methods used to suppress these pests.

Estimates on crop losses are pre-requisite for implementation of management practices against vertebrate depredations. There are

several methods used for assessing crop losses due to vertebrate pests. Parameters used for assessing crop-losses vary from one investigator to another, thus rendering the data often uncomparable (Figs. 7-18) provide a glimpse of the extent, symptoms and nature of damage by the vertebrates and the plant part (s) exposed for infection. Generally the disease is confined to a tree or few surrounding trees. But the entire orchard may also be affected by the disease as a result of vertebrate damage. Published information on crop losses due to diseases by a vertebrae could not be found. For instance, in orchards, losses are caused by vertebrates from flowering to the ripening stage of the fruits. Usually many flowers are consumed, the unripe fruits are ribbled and dropped from the tree and then the ripe fruits are scooped from the inside an large number are broken off the branch and dropped to the ground. This type of damage is sever in some parts of the country and cultivation of the crop is abandoned altogether.

There is an urgent need to standardize the methods of assessing losses due to vertebrate pests. In rodents, birds, and desert ecosystem vertebrates some progress have been made. A synthesis of all these scattered informations available on various aspects of vertebrates like distribution, population fluctuation, pest management, *etc*. in the country should be made. After all control measures are to be based on a proper translation of ecological factors into management policy (Ishwar, 1968). A nodal agency can constitute a committee of experts to find gaps in research and set priorities for zone-wise research on vertebrate pests, for the 21st century.

The Vertebrate Pest Manager should not aim at eliminating a specific community of animals to realise cent per cent crop protection. For instance, in cocoa plantations in Sringeri, Chickmagalaur and Thirthahalli, Shimoga rats and squirrels were removed, but jungle cats occupied the same niche. When jungle cats were exterminated from the patch, jungle crows devoured the contents of pods in a major way. When crows were scared away monkeys, *M.radiata* occupied the niche. Thus a succession in the community of animals depredating cocoa pods. Similarly, application of chemicals against damage of vertebrates may result in biomagnification. Total protection may lead to consequences difficult to monitor in the ecosystem.

Fig. 7 : Bat damage to grape bunch

Fig. 8 : Bison damage (lodging) to guava tree

Fig. 9 : Bird (Parakeet) damage to guava fruits

Fig. 10 : Bison damage (debarking) to sapota tree

Fig. 11 : Elephant damage (debranching) to Sapota tree

Fig. 12 : Bird (Small green barbet) damage to banana fruits

Fig. 13 : Wildboar damage to mango fruits

Fig. 14 : Rat Damage to coconuts

Fig. 15 : Monkey damage to cocoa pods
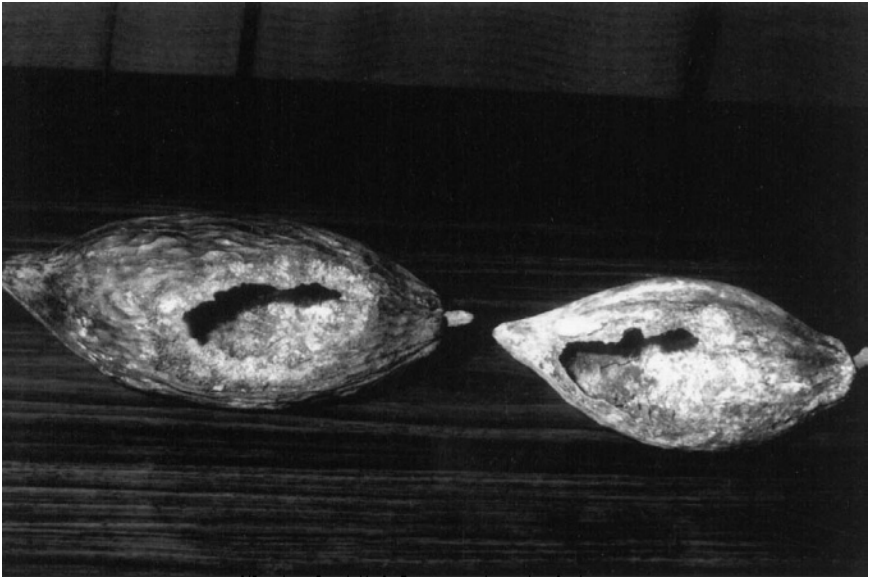
Fig. 16 : Jungle cat damage to cocoa pods

Fig. 17 : Monkey damage to cashew apples

Fig. 18 : Porcupine damage to cashew nuts

7. Priorities

Novel tools for vertebrate pest management in cultivated systems in India can include solar power operated fence, plant/animal products, provision of alternate or known natural foods for vertebrates in the vicinity of agro-ecosystems, trenches, cultural practices that do not involve much labour such as polyculture, timely harvests, lacing repellent pastes made of easily available materials and mechanical barriers made of locally available, biodegradable and renewable materials.

Use of botanicals especially in the evergreen tropical forest tracts have considerable potential (Bhat *et al.,* 1995, Bhatnagar, 1982, Bhatnagar *et al*., 1993). In fact botanicals serve as the ideal tools for Integrated Vertebrate Pest Management (IVPM). But little is done in this regard. Neem, cluster beans, agave, tree bark decoctions and species of Acacia are plants worthy of investigation (Bindra and Toor, 1972).

It is not always necessary to use chemicals to protect crops from vertebrates. Baiting is the most common method adopted against rodent pests (Gooding, 1961). While horticultural products should find way into international markets devoid of any chemical residues, products slightly damaged by vertebrates can find a place in local markets, picking, canning industries and in confectioneries. Public in general, in India should tolerate little damage by vertebrates in view of the ecological and environmental roles vertebrates play in different ecosystems.

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Section 2
Vegetable Diseases

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5

Nutrient Deficiency Disorders in Vegetables and their Management

C. Chatterjee and B.K. Dube

ABSTRACT: Among the horticultural crops vegetables have an important position and is a high protective food of dietary complex of human beings. For balanced diet suplementation of vegetables along with cereals and pulses is a necessary step towards complete food. In recent past the production of vegetables have gone up due to adaptation of modern technology and fertilization formulation but still do not show any parallelism with consumption. For sustainable production, the vegetable crops exert tremendous pressure on the soil for nutrients due to their productivity ability. This results in depletion of essential nutrients from the soil. To evaluate fertility status of soils several techniques are in vogue. In addition to visual symptoms of each essential nutrient for various crops their critical concentrations have also been worked out for most of the vegetables. Soil analysis further substantiate these findings for actual nutrient status. In certain cases when visible symptoms due to any deficiency is not perceptible, or the plant shows latent deficiency help of biochemical parameters are also useful.

1.   Introduction

Vegetables among horticultural crops are major potential crops for meeting the food requirements of the people. Vegetables are rich sources of essential nutrients, play a significant role in improving the nutritional status of mal-nourished people whose diet are mainly based on cereals. Besides this, vegetables have many agro-economic advantages and can fit into varying cropping systems under diversified conditions.

The vegetables are recognized as natural resources of productive food and comparatively cheaper source of vitamins, mineral and dietary fibers (Table 1). The demand for vegetables is increasing everyday owing to increase in population and growing awareness among the people regarding the nutritional importance of vegetables in human diet.

At present India is the second largest producer of vegetables in the world after China. In last two decades, the production of vegetables have gone up significantly, which could be possible because of judicious use of vegetable production resources by adoption of scientifically sound agronomic practices involving high yielding genotypes, increased use of NPK fertilizers, decreased use of organic manures and lack of recycled crop residue.

All these activities might create a situation that the inherent pool of most of the metals in soils are being gradually depleted. This in turn would cause several disorders including that of nutrient deficiencies resulting in lowering of yield and quality of the crops and remain major constraint for high and sustainable vegetable production.

2.   Nutrient Requirement of Vegetable Crops

Nutrient requirement is basically a genetic characteristic of the crop plants and this requirement may vary with the genotypes of particular crop. Nutrient removal by any crop depends on the nutrient availability in soils and their absorption, which is influenced by soil pH, moisture and temperature. In general vegetable crops exert tremendous pressure on the soil for nutrients due to their high productive ability. Nutrient removal is perhaps the most critical when sustainability of a farming system is considered. If the nutrient removal from the fields is not replaced, the farming system will not remain sustainable due to decline in the soil productivity.

Vegetables among protective foods are rich sources of essential elements besides having medicinal and therapeutic properties and are able to provide nutritional security to predominating vegetative country.

The beneficial effects of addition of fertilizers including organic matter containing mineral nutrients, to soil for improved growth of plant is known in agriculture from time immemorial.

This information was first reported by Justus Von Liebig [1803-1873] who established for the first time the importance of mineral elements for plant growth (*see* Hewitt, 1957). Plants have a restricted capacity for the selection of uptake of nutrient elements, which are

TABLE 1
Nutritive value of different vegetables

Nutritive value of different vegetables
(Per 100 g edible portion on fresh weight basis)

S.No	Name of vegetables	Moist (g)	Carboh-ydrates (g)	Protein (g)	Fat (g)	Calorie energy	Vit.A (IU)	Thia-mine (mg)	Riofl-avin (mg)	Ascorbic acid (mg)	Ca (mg)	Fe (mg)	P (mg)
1	Potato	74.7	22.6	1.6	0.1	97	40	0.40	0.04	17.0	10.0	0.7	35.0
2	Tomato (ripe)	94.0	3.6	1.2	0.1	20	302	0.12	0.06	27.0	48.0	0.4	26.0
3	Chilli	85.7	3.0	2.9	0.6	29	292	0.19	0.39	111.0	30.0	1.2	80.0
4	Brinjal	92.7	4	1.4	0.3	24	118	0.04	0.11	12.0	18.0	0.9	47.0
5	Cabbage	92.4	5.3	1.4	0.2	29	80	0.06	0.05	100.0	46.0	0.8	38.0
6	Cauliflower	91.7	4.9	2.4	0.2	31	70	0.04	0.03	75.0	30.0	17.0	76.0
7	Knolkhol	90.1	6.7	2.1	0.1	36	20	0.05	0.1	50.0	20.0	0.4	60.0
8	Broccoli	89.9	5.5	3.3	0.2	37	3500	0.05	0.12	137.0	80.0	0.8	79.0
9	Bitter gourd	92.4	4.2	1.6	0.2	25	210	0.07	0.09	88.0	20.0	1.8	70.0
10	Pointed gourd	92.0	2.2	2.0	0.3	20	255	0.05	0.06	29.0	30.0	1.7	40.0
11	Pumpkin (ripe)	86.0	4.6	1.4	0.1	25	2180	0.06	0.04	2.0	10.0	0.7	30.0
12	Raddish (White)	94.4	3.4	0.7	0.1	17	50	0.06	0.02	17.0	50.0	0.5	20.0
13	Carrot	82.2	10.6	0.9	0.2	48	12000	0.04	0.02	3.0	48.0	0.6	30.0
14	Turnip	91.6	6.2	0.5	0.2	28	4	0.04	0.04	43.0	30.0	0.4	40.0
15	Onion	86.8	11.0	1.2	0.2	50	35	0.08	0.01	11.0	180.0	0.7	50.0
16	Garlic	62.8	29.0	6.3	0.1	142	10	0.16	0.23	13.0	30.0	1.3	310.0
17	Shallot			2.6				0.06	0.02	1.0	37.0	1.3	60.0
18	Lettuce	93.4	2.5	2.1	0.3	21	540	0.09	0.13	10.0	50.0	2.4	28.0
19	Okra	89.6	6.4	1.9	0.2	35	88	0.07	0.1	13.0	66.0	1.5	56.0
20	Pea	72.0	15.8	7.2	0.1	93	300	0.25	0.01	19.0	20.0	1.5	139.0
21	French bean	91.4	4.5	1.7	0.1	25	321	0.08	0.06	16.0	50.0	1.7	28.0
22	Cowpea	84.6	8.0	4.3	0.2	51	941	0.07	0.09	13.0	80.0	2.5	74.0
23	Cluster bean	81.0	10.8	3.2	0.4	59	316	0.09	0.09	47.0	130.0	5.0	50.0
24	Broad bean	85.4	7.2	4.5	0.1	48	14	0.08		12.0	50.0	1.4	
25	Drumstick pod	89.6	3.7	2.5	0.1	25	176	0.05	0.07	120.0	30.0	3.3	110.0
26	Palak	86.4	6.5	3.4	0.8	46	9770	0.26	0.56	70.0	380.0	16.2	30.0
27	Water spinach	92.4		1.9			4800			58.0	90.0	4.8	
28	Fenugreek leaves	86.1	6	4.4	0.9	49	3744	0.05		54.0	360.0	17.2	51.0
29	Mustard leaves	89.8	3.2	4.0	0.6	34	4195	0.03		33.0	155.0	16.3	26.0
30	Bottle gourd leaves	87.9	6.1	2.3	0.7	40	0				80.0		59.0
31	Coriander leaves	86.3	6.3	3.3	0.6	44	11168	0.5	0.06	135.0	184.0	18.5	0

Based on Thomson and Kalley 1959; Nath 1976; Bernad 1979; Bradry 1980; Bose and Some 1986; Ghosh *et al* 1988; Korokov and Kiram 1988; Peter and Devadas 1989; Shaumjavelu 1993; Indira and Peter 1993

essential for their growth. Apart from this, plants also take up those mineral elements, which are not essential and sometimes may be toxic also. Several techniques have been worked out for establishing the essential role of specific nutrients. On the basis of these observations essentiality of nutrients was established. To define an essential element three criteria of essentiality has been specified by Arnon and Stout (1939) *viz*, (i.) omission of element causes failure of growth or reproductive processes. (ii.) the element cannot be replaced by another

element in these or all respects. (iii.) the element is associated with an essential metabolite (Hewitt, 1983).

Further studies have shown that certain elements act as beneficial elements depending on their beneficial effects and extent of requirement. Therefore, these three criteria of nutrient essentiality cannot be generally applied.

For higher plants essentiality of sixteen mineral elements are well established. With continuous improvements in the analytical techniques the list was extended to include mineral elements that are essential only in very low concentration for plants (*i.e.*, that act as micronutrients). The essential mineral nutrients have been classified into two major groups:

2.1. Macronutrients

The nutrients which are required by plants in large quantities (> 1 ppm) are called as macro or major nutrients. These are nine in number, such as nitrogen, phosphorus, potassium, (primary nutrients), calcium, magnesium, sulphur, (secondary nutrients) in addition to carbon, hydrogen, and oxygen. The macronutrients are either constituents of organic compounds such as proteins and nucleic acids or are involved in maintaining osmotic pressure.

2.2. Micronutrients

The elements which are required by plants in small quantities (< 1 ppm) are also known as minor or trace elements, *e.g.*, iron, manganese, copper, zinc, boron, molybdenum, and chlorine. Most of the micronutrients are predominately constituents of enzyme molecules and thus are essential in small amounts. The differences in function between that of macro- and micronutrients may also determine the average contents required for sufficient or adequate quantity by plants.

The mechanism by which plants take up nutrients is rather selective. The selectivity depends not only on genetic characteristic but also dependent on physical and chemical properties of plants along with its essentiality.

In higher plants like vegetables and other field crops when the concentration of an available essential nutrient is abnormally high or

low, characteristic visible symptoms appear on any part of the plant, which can be of help in diagnosing the disorder due to either low concentration or omission of a particular essential element or high concentration within the plant.

On the basis of visible symptoms diagnosis of deficiency and toxicity of elements (essential and non-essential) is a simple and quickest method for determining the causes of crop failure. Illustrated account of deficiency symptoms of micronutrients specially have been brought out by Cook and Miller (1953), Viets *et al.* (1954), Olsen (1958), Wallace (1961), Stiles (1961), Hewitt (1963), Sprague (1964), Chapman (1966), Tanaka and Yoshida (1970), Bergman and Neubert (1970) and Bergmann (1992). These have been helpful in diagnosis of micronutrient deficiencies from different parts of the world.

The micronutrient concentration within the plants reflects the availability of the nutrient in the rooting medium and may serve as an index for its status in terms of deficiency, sufficiency or toxicity. When the tissue content of a micronutrient is calibrated in terms of yield, it is possible to specify the concentration indicative of even such an extent of deficiency as may limit plant yield but is not large enough to induce visible symptoms, a condition often referred to as "hidden hunger". The identified values are called as critical limits.

Sometimes the visible symptoms of nutrient deficiency are advanced by the presence of several non-nutritional factors such as growth regulators, herbicides, sprays, some pests and insect diseases and air pollutants. In certain other cases if more than one nutrient is deficient in plants quiet different symptoms appear caused by low concentration or absence of not only of one but may be due to more than one deficiency. Under these circumstances a deficiency by visible symptoms alone can be misleading. If all these could be identified then fertilization of the vegetables fields could be easier and precise.

3.   Management of Nutritional Deficiencies

Diagnosis of nutrient deficiencies in the assessment of fertilizer requirement of vegetables can be done by several methods including:

1.   Diagnosis through characteristic visible symptoms of individual nutrient deficiency

2. Experiments under controlled conditions and field trials
3. Soil analysis
4. Plant tissue analysis
5. Assessment of nutrient status of plants by biochemical parameters.

3.1 Characteristic Visible Symptoms of Nutrient Deficiency

The visible effects of nutrient deficiencies (Table 2) in several instances are related to their function in plant metabolic systems or no connection is observed with these systems because several effects may be involved and secondary changes may also occur. But here is a description involving characteristic effects on vegetables of various mineral disorders which will be helpful in diagnosing the diseases/disorders under field conditions.

TABLE 2
Some principles of visual diagnosis of nutritional disorders

Plant Part		Prevailing Symptom	Disorder
			Deficiency
Old and mature leaf blades	Chlorosis	Uniform	N (S)
		Interveinal or blotched	Mg (Mn)
	Necrosis	Tip and marginal scorch	K
		Interveinal	Mg (Mn)
Young leaf blades and apex	Chlorosis	Uniform	Fe (S)
	Necrosis	Interveinal or blotched	Zn (Mn)
			Ca, B, Cu
	Deformations		Mo (Zn, B)
			Toxicity
Old and mature leaf blades	Necrosis	Spots	Mn (B)
		Tip and Marginal Scorch	B, salt (Spray, injury)
	Chlorosis		Nonspecific toxicity

3.1.1. Nitrogen
Nitrogen plays a vital role by affecting physiological activities in various ways. It is a component of protoplasm, chlorophyll molecules, nucleic acids and amino acids, of which proteins are made. In building up of the plant body nitrogen

associates with several other macro- and micronutrients. Nitrogen compounds constitutes 40-50 % of the dry matter of protoplasm.

Nitrogen Deficiency

At the initial stages the development of symptoms is very slow in the form of slight yellowing from near the edges of lamina of old leaves. These symptoms are accompanied by depression in growth. In acute deficiency severe angles between petioles and the stem in certain broad leaved species *e.g.* tomato and potato are formed. In other plants, yellowing of green stem also occur. The yellowing usually starts from old leaves which gradually changes to necrosis and later the entire plant may turn brown in severe deficiency. Foliage is pale green as leaf senescence and dehiscence are accelerated. Leaves often develop strong purple, red or orange pigmentation especially in brassicas (cauliflower, cabbage *etc.*) where yellowing of plants is accompanied by loss of chlorophyll, and appearance of supplementary pigments. Nitrogen deficiency also hastens maturity. Nitrogen deficient plants have high flower drop especially at high temperature when the plants are starving due to increased transpiration rate.

3.1.2. Phosphorus

The main function of phosphorus is either that of energy transfer or formation of pyrophosphate anhydride bonds in nucleotides. It is also involved in the formation of nucleic acids. Phosphorus as phosphates of several compounds is involved in different metabolic systems, it plays a specific role in the formation of activated amino- acyl tRNA molecules. It is also present in proteins, phytic acids and in several phosphorus storage products, as phospholipids associated with membrane proteins (Mazliak, 1973) and in maintaining the membrane structure.

Fig. 1:   Phoshorus deficiency in tomato : Plants thin and weak, old leaves chlo-rotic deep yellow, hanged downward, dry, withered, petiode form acute angle with stem, poor fruit formation.
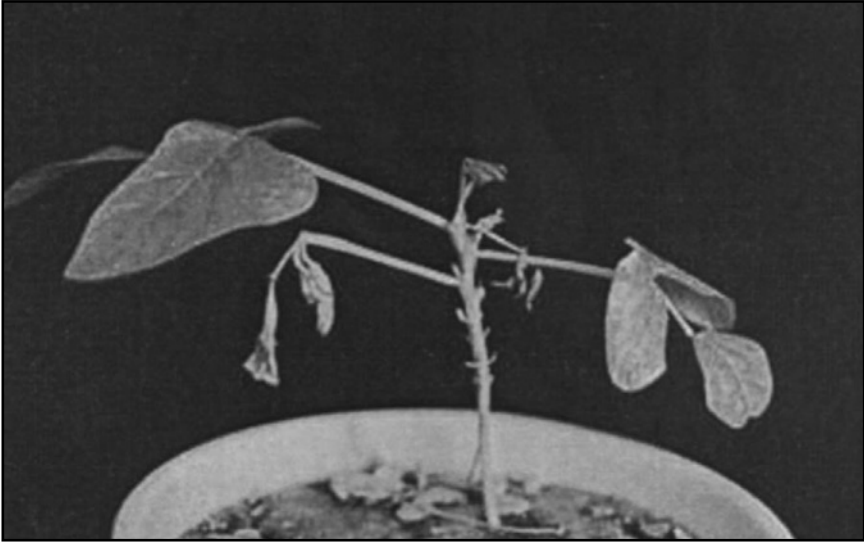
Fig. 2: Acute phosphorus deficiency in Cowpea: Plant short in size, old leaves dry and wither, upper leaves chlorotic at margins, chlorosis spread from old to young leaves.

Phosphorus Deficiency

Stunned growth, the younger leaves are dark green and old leaves develop purple or reddish anthocyanin pigmentation. In phosphorus deficiency the old leaves have a tendency to turn dull, bluish green. Plants are thin erect and leaves are narrow with stunted petioles. (Figs. 1,2)

The vegetables those are highly susceptible to phosphorus deficiency are-carrot, lettuce, spinach, french bean, onion, and turnip.

Less susceptible are Cabbage, Cauliflower, Parsnip, Pea, Raddish, etc

3.1.3. Potassium

Potassium is an important cell base ion and required for balancing the negative charge of organic acids produced within the cell and anions. It is involved as an activator of several enzymes. In plants protien synthesis is mainly dependent on potassium at several stages of amino acid activation. Another important function of potassium is in the control of stomatal aperture by the movement of guard cells (Fujino 1967, Fisher and Hsiao 1968, Humble and Raschke 1971). Potassium is indirectly involved in the process of ion transport across membranes in which most of the ATPase systems are probably located. Potassium maintains plants against cold and drought.

Potassium Deficiency

The growth of plants depressed, development of lesser number of branches and leaves. Marginal scorching of old leaves, scorched margins, curl up or down, shortened internodes, wilting and early abscission. Diffused interveinal chlorosis

of old leaves, later entire leaf appears yellow, marginal scorching may be preceded by marginal chlorosis, with small brown irregular spots, later the spots enlarge, coalesce and cover larger interveinal areas. In several plants the affected leaf fall off in persistent deficiency. (Figs. 3,4).
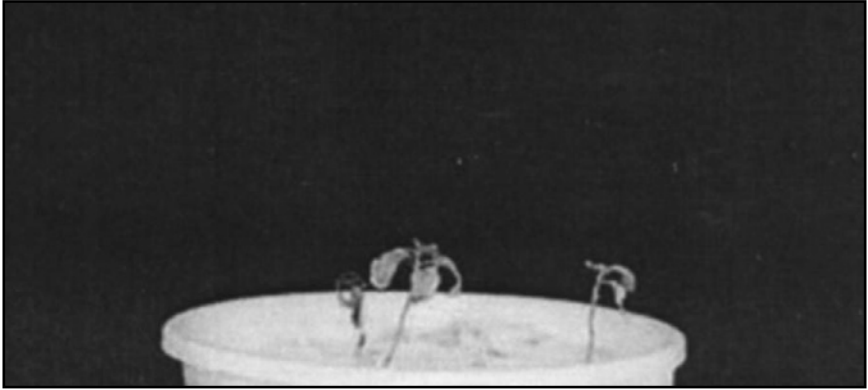
Fig. 3.   Potassium deficiency in Chilli: Growth of plants highly restricted, old leaves chlorotic, bend downward, gradually turn necrotic and dry.

Fig. 4.   Potassium deficiency in French bean: Plants distorted, reduction in leaves size, all leaves appear yellow, leaf apex and margins scorched, curl downward, veins green, old chlorotic leaves dry and collapse.

The maximum susceptible species is spinach, fairly susceptible are broad bean, brocoli, cauliflower, lettuce, onion, raddish, turnip. Whereas cabbage, carrot, parsnip, pea are less susceptible to potassium deficiency.

3.1.4. Calcium

Calcium plays a vital role in the structure, stability and formation of membranes and is involved in maintenance of nucleus and chromatin (Hewitt 1963, Burstrom 1968, Epistein 1972, Gauch 1972, Rains 1972, 1976). In calcium deficiency chromosomes fail to separate completely, cell plate is not formed, spindle is abnormal and chromatin is aggregated. Cell organelles with limiting membranes, less in number, distorted and disintegrated with perforated membranes. It is required to maintain retention within or transport of potassium across membranes. Calcium is involved in cell wall composition in the form of Ca-salts with pectic acid, in cell division. Calcium has been suggested to control germination and direction of growth of pollen cell tube (Gauch, 1972) probably in association with boron.

Calcium activates several enzymes, *e.g.*, phospholipases, several ATPases, enolases etc. (Davidson and Long 1958, Dodds and Ellis 1966, Paulsen and Harper 1968), induces the activity of nitrite reductase in cucumber leaves. In addition calcium has several indirect roles in plant metabolism.

Calcium Deficiency

The deficiency symptoms of calcium normally appear on younger leaves and near the growing regions of stem and roots. In addition to growth depression, the
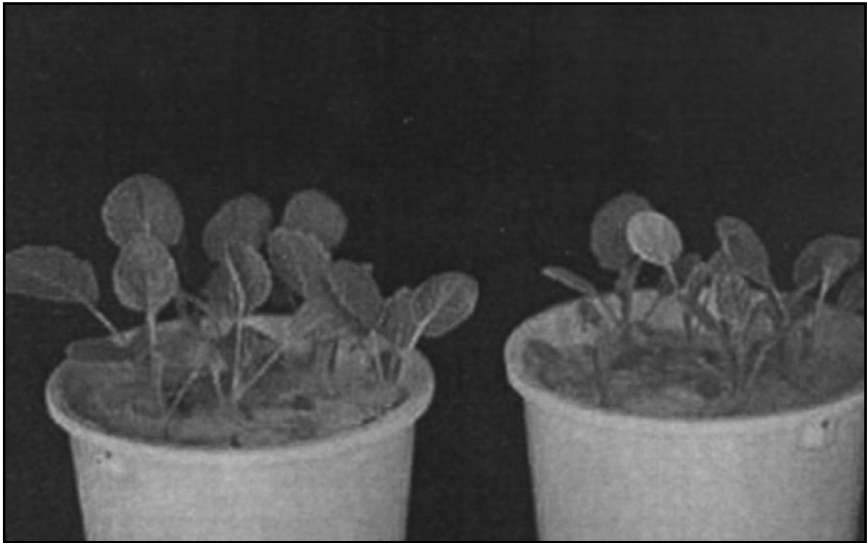
Fig. 5.   Calcium deficiency in cauliflower: From left to right: A pot with normal plant (L), a pot with deficient plant (R). Growth of plant and leaf size reduced, Interveinal chlorosis of young leaves, chlorosis initiating from apical margins, young emerging leaves show cupping.
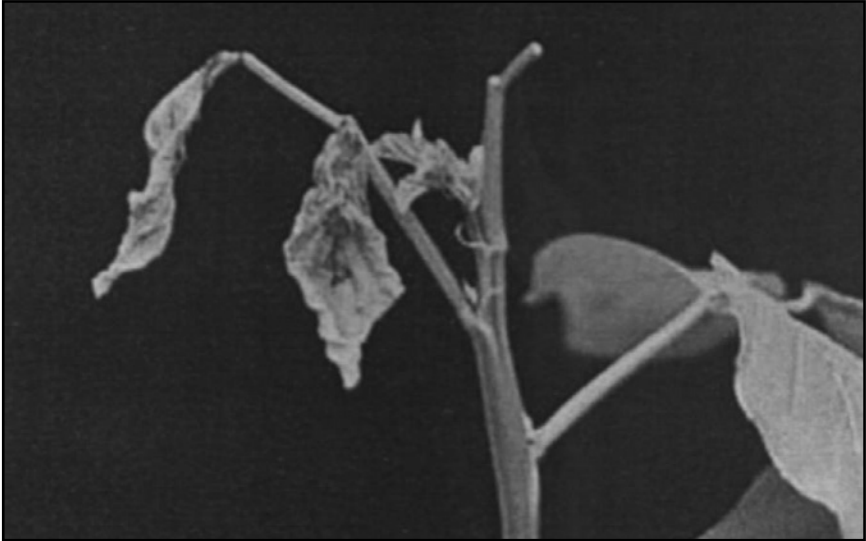
Fig. 6.   Acute Calcium deficiency in cluster bean: Young leaves severely chlorotic, deformed, necrotic, dry and collapse, premature leaf fall, growth checked due to complete loss of apical shoot.

leaves appear smaller in size. The affected young leaves show cupping (both inward and outward) tipburn or extensive blackening at apical region. Pale marginal band develops on young leaves. In severe calcium deficiency, appearance of water socked areas, on interveinal areas, the affected leaf appears flaccid, later collapses from petiole or stem (joint). In deficiency, young leaves may show distortion of tissue from interveinal areas and form a hole due to complete loss of tissue. In some cases burning and necrosis of margins of leaf occur (Figs. 5,6). Specific names have been given to several diseases that are caused by calcium deficiency in vegetables e.g., lettuce tipburn, internal burning of brussels sprouts and blackheart of celery etc.

3.1.5. Magnesium

Magnesium is a constituent of both chlorophyll a and b which contains 3.7% of magnesium and together represent approximately 10% of the total leaf magnesium. Many of the enzymes present in chloroplast involved in photosynthesis required magnesium as a dissociable activator. In the deficiency of magnesium the chloroplast structure undergoes early derangement (Thomson and Weier 1962, Marinos 1963, Vesk *et al*. 1966, Whatley 1971). Several reports suggest (Marinos 1963, Hewitt 1983) that mitochondria which contain several magnesium dependent enzymes also undergo structural degeneration when deficient in magnesium. It plays an important role in the stability of ribosomal subunits. The association of subunits of ribosomes is controlled partly by magnesium. Presence of magnesium is essential for the binding of transfer RNA – aminoacyl acid complex to the ribosome.

Several enzymes of carboxylic acid metabolism require Mg as an activator and in certain instances magnesium is replaced by manganese . The chloroplasts in Mg deficient leaves develop larger starch grains. Grana remains greatly reduced in size, irregular and vacuolated and sometimes lose chloroplast membranes, as a result the contents are dispersed in the cytoplasm (Hall *et al*, 1972).

Magnesium Deficiency

The visible symptoms of low magnesium appear late on old leaves as loss of green colour from interveinal areas. which is followed by bleaching of the affected leaves. With increase in age, symptoms turn severe, necrotic lesions appear irregularly near the edges of the affected leaves. This consequently gives a rugged appearance to the leaf, ultimately in severe conditions the leaf hangs down due to the formation of abscission line from end of the petiole. In several vegetable plants, premature defoliation usually occurs. In some plants *e.g.* peas, tomatoes *etc*. the green margin often turns yellow or develop brilliant orange red or purple tints. Similar tints also occur generally on the mottled areas in chlorotic leaves *e.g.* in cauliflower, broccoli *etc*.

In several instances the effects of magnesium deficiency are difficult to distinguish form that of severe potassium deficiency *e.g.* in lettuce, vegetable marrow (Shorrocks 1964) or in dwarf varieties of french beans (Fig.7). On affected old leaves, necrotic brown lesions / spots appear on the bleached areas, later coalesce into larger scorched areas. The leaves become generally bright pale green or yellow green and with increase in age these leaves appear totally bleached (Fig.8).

Fig. 7.   Magnesium deficiency in French bean: Interveinal chlorosis of old leaves more towards edges, chlorosis initiating from margins and covered entire lamina, chlorotic areas developed irregular brown necrotic spots.
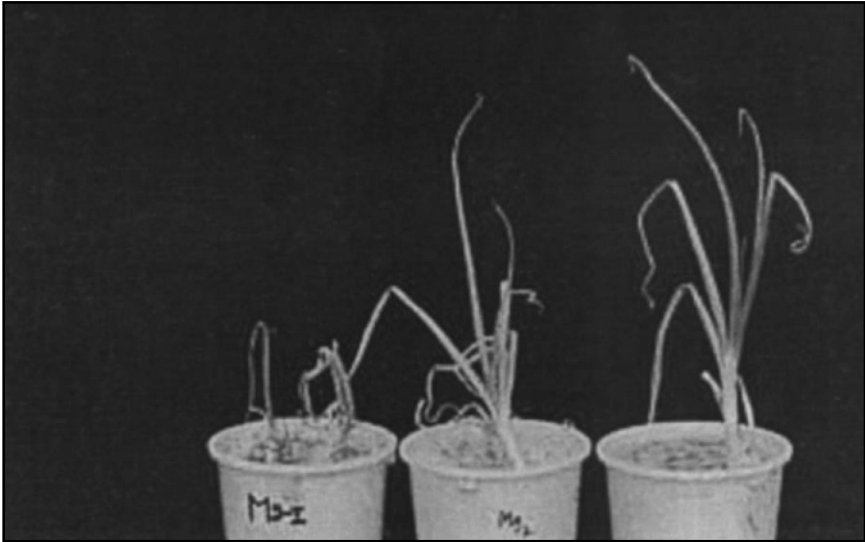
Fig. 8.   Magnesium deficiency in onion: From left to right: (R) normal plant, (centre), moderate deficiency, (extreme left) acute magnesium deficiency. Growth of deficient plants depressed, apices of old leave pale yellow, necrotic and dry.

3.1.6. Sulphur

The role of sulphur is important as it is incorporated into two amino acids – cysteine and methonine. These amino acids are precursor of other sulphur containing compounds such as co-enzymes and secondary plant products. About 2% of the organic reduced sulphur in the plant is present in the water soluble thiol (- SH) fraction. One of the S-containing organic compounds *e.g.* glutathione is important as it serves many functions in plants.

In chloroplasts thioredoxins function primarily as regulatory proteins in carbon metabolism. This is another S containing biomolecule involved in plant system. Reduced sulphur is a structural constituent of several co-enzymes and prosthatic group such as ferredoxin. Glucosinolates are characteristic compounds of secondary metabolism containing sulphur. More important compounds such as alliins and glyucosinolates – are of particular relevance for horticulture and agriculture (Schung 1993). The role of secondary compounds containing sulphur has not been understood properly but their defensive role is well known (Ernst 1993). In chloroplast membranes sulphur in the form of sulpholipids are abundant (Schmidt 1986) which are usually involved in the regulation of ion transport across biomembranes. Sulpholipid level in roots is positively correlated with plant salt tolerance.

Sulphur Deficiency

In addition to depression in growth, the visible effects appear on young leaves as interveinal chlorosis or yellowing, starting from margins and apical end. In severe

deficiency the affected leaves turn golden yellow, and the symptoms travel fast on the lower leaves. The thickness / diameter of stem and number and size of leaves are highly reduced and deformed. The affected leaves sometimes form spoon like or cup like structure, foliage appears stiff and plant remains erect. The roots and stem become abnormally long and develop woodiness.

More susceptible species are cabbage, radish, turnip. Fairly susceptible are broccoli, cauliflower, lettuce, onion, pea, spinach. Less susceptible are broad bean, carrot, celery, french bean, parsnip.

3.1.7. Iron

Iron has been reported to be essential for diverse group of plants (Marschner 1995). The requirement of iron has been found to be different for different crops. Iron has an erratic position among essential nutrients because it behaves like a macro- or micronutrient depending on great variation in its requirement by different plant species. Iron plays an important role in chloroplast development and maintenance of its integrity (Jacobson 1945, Terry and Low 1982). On the other hand, iron is supposed to play a possible role in the synthesis of some specific RNA that regulate chlorophyll synthesis. Iron deficiency has been reported to disturb development of chloroplast. Retarded and abnormal development with reduced number of stroma in chloroplasts are common features in low iron (Spiller 1980, Ji *et al* 1984, Kaleya *et al* 1989), Nishio *et al* (1985) demonstrated that in iron deficiency the synthesis of thylakoid galatolipids as well as proteins are depressed. Low iron is known to decrease the synthesis of P 700 molecules and thus lowering the primary electron acceptor complex of PSI. iron is involved in several heme and non-heme compounds. Many enzymes and electron carriers contain a heme prosthetic group (Marschner 1995). In plants, animals and bacteria a special class of proteins contain non-heme iron bound to sulphur atoms. The more important enzymes containing Fe are those which bring about oxidation - reduction reactions in plants. It regulates respiration, photosynthesis, reduction of nitrate and sulphates.

Iron Deficiency

In iron deficiency young growth is usually affected, interveinal chlorosis of upper leaves initiates from apex and gradually travels downward. The pale chlorosis turned progressively yellow or white (bleached) as the deficiency becomes more serious (Figs. 9,10). No distinct deformity occurs but development of leaves and branches are restricted. In acute deficiency brown necrotic spots may occur on the bleached foliage. In severe deficiency all trace of green colour on the youngest leaves is absent leaving a strikingly white leaf. In acute persistent deficiency, the growth of plant is highly depressed. The number and size of leaves are reduced (both affected and non-affected) in persistent iron deficiencies where the economic is also disturbed.

Fig. 9. Acute iron deficiency in radish: Young and middle leaves chlorotic and bleached with clear veins.
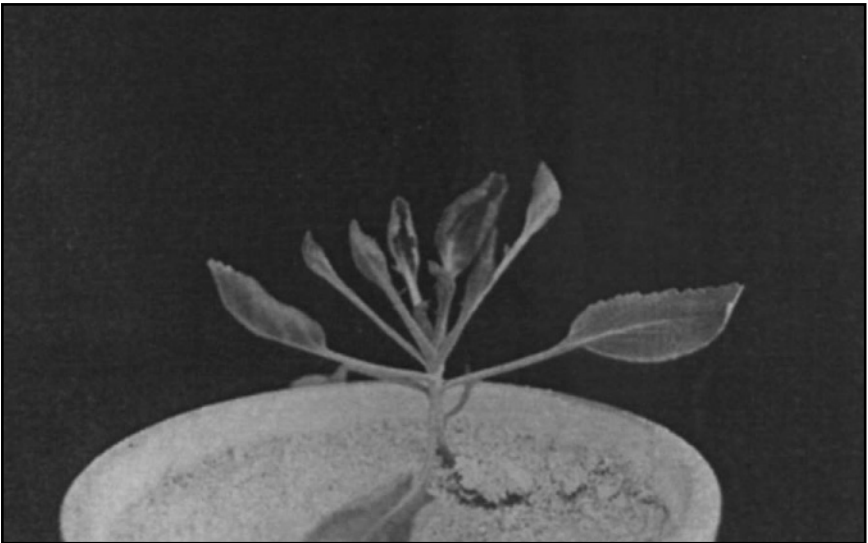
Fig.10. Iron deficiency in cauliflower: A pot with deficient iron. Growth of plant and leaf size reduced. Entire plant appears bleached, leaves dry.

3.1.8. Manganese

Manganese activates several enzymes. The requirement of Mn is highly specific in photosynthesis (Pirson 1937, 1958, Cheniae and Martin 1966, Kok and Cheniae 1966, Cheniae 1970) as well as in auxin oxidase system (Hewitt 1957, 1963, Yamazaki and Piette 1963, Fox *et al*. 1965, Hinman and Long 1965, Schneider and Wightman 1974). Mn is involved in the regulation of respiration and protein synthesis. It substantially acts in the protection of membranes against lipid peroxidation (Thiele and Huff 1960)

Manganese Deficiency

The plants show depression in growth and usually visible effects appear on middle and young leaves**.** The symptoms of Mn deficiency initiate late as interveinal chlorosis of fully mature young leaves. The chlorosis spread to middle leaves with increase in age of plants. Irregular white necrotic spots develop on the chlorotic portion of the sub-terminal leaves (Figs. 11,12). These spots later enlarge in size and spread to the entire lamina, but is most marked at the margins, which wither after turning severely necrotic. In severe deficiency of Mn, the necrotic spots enlarge in size, coalesce and entire leaf later turn dry, necrotic and wither.

Owing to Mn deficiency on seeds of pea, beans etc. some irregular necrotic spots develop and gradually turn deep brown in colour. In persistent deficiency, the seeds split open. This disease/disorder has been named as "Marsh spot of Pea". In spinach the middle and young leaves show loss of lamina and as a result they appear arrow like.

Fig.11. Manganese deficiency in radish: Interveinal chlorosis and mottling of young and old leaves; lamina bend backward.

Fig.12.   Manganese deficiency in French bean - Interveinal chlorosis of middle leaves more towards edges. Chlorosis initiating from edges, veins green.

3.1.9. Copper

In Cu deficiency concentration of chlorophyll and activity of photosynthetic electron transport system are decreased . The role of Cu in one of the enzymes *i.e.* ribulose-biphosphate has been contributed to a specific protein (Branden 1978). Copper is also involved as a constituent of plastocyanin in the photosynthetic electron transport system. Several enzymes with many diverse properties and functions are dependent on copper which is tightly bound to the protein *e.g.* phenolases, ascorbic acid oxidase, cytochromes and cytochrome oxidase, superoxide dismutase *etc*. (Pridham 1963, Brill *et al*. 1964, Peisach *et al*. 1966, Malkin and Malmstrom 1970, Hewitt and Smith 1974).

Copper Deficiency

The visible effects of low copper appear comparatively late on young growth. The size of upper leaves is reduced and often patchy discolouration on the lamina of these leaves are discernible. The margins of affected leaves curl inward (Figs. 13,14). In pea, the size and thickness of tendrils are markedly reduced and appear pale from tip. With persistent deficiency the young growth show sever discolouration or bleaching and as a result of this premature drying with ceasation of terminal growth occurs. Further growth and coiling habit of the tendrils is restricted. The appearance of Cu deficiency symptoms in vegetables vary with the species *e.g.* in carrot, no distinct deficiency symptoms appear but the loss in yield is very marked. In acute deficiency, the young leaves are severely bleached and necrotic brown or grey or blackish spots appear on the affected portion.

Fig.13. Copper deficiency in French bean: Growth of plants depressed due to short internodes. All the leaves show interveinal chlorosis, young leaves bleached, dry and turn backward.

Fig.14. Copper deficiency in okra: (L) normal plant (R) deficient plant, short number and size of leaves reduced, middle and old leaves with interveinal chlorosis, the affected leaves bend downward, plants gives ragged appearance.

3.1.10. Zinc

The metal is involved in several enzymes of living system such as dehydrogenases, proteneases and peptidases. Zinc is required for the synthesis and utilization of carbohydrates in plants. In zinc deficiency the concentration of amides and total amino nitrogen increases in higher plants. The concentration of soluble proteins

is known to decrease in low zinc conditions. It has a specific role in nucleic acid metabolism, and plays an indirect role in the synthesis of chlorophyll and also affects photosynthesis when present in low concentrations. Zinc is a component of the enzyme, carbonic anhydrase which acts in the transfer of CO_2 through the liquid phase of the cell to the chloroplast surface (Hatch and Slack 1970). In several crops such as tomato, spinach and beans, there is a close relationship between concentration in leaf tissue and carbonic anhydrase (Wood and Silby 1952, Bar-Akiva and Lavon 1969, Edwards and Mohamed 1973, Randall and Bouma 1973, Ohki 1976). The rate of photosynthesis is reduced in low zinc as a close relationship occurs between photosynthesis and the enzyme activity. It has been suggested to be an indicator of zinc status of plants. It plays an important role in reproductive physiology of higher plants.

Zinc Deficiency

In general, zinc deficiency results in shortened internodes and interveinal chlorosis of middle and mature leaves is a common feature. The visible effects of low zinc appears early (after 2 weeks growth) except where growth in early stages is very slow. Owing to shortening of internodes, a large number of lateral branches with leaves arise in a rosette like manner, the new as well as emerged leaves (young) are markedly reduced in size with short petioles giving the plant a bushy habit (*e.g.* beans, pea *etc.*). In sever deficiency growth of plants is highly depressed, flowering is early but most of the flowers are shed premature. In persistent and prolonged zinc deficiency, interveinal chlorosis on the affected mature young or middle leaves intensifies, margins of the affected leaves are scorched and limp down and the chlorotic lesions change to necrotic with increase in age of the plants (Fig. 15).

Fig.15. Zinc deficiency in brinjal: (L) normal plant (R) deficient plant. Depressed growth, small chlorotic leaves (more towards apical end), no flower or fruit.

Beans are very susceptible to zinc deficiency. According to Scaife (1988) the plants appear pale green, chlorosis appears between veins and leaf tips and edges. The emerging leaves are deformed, dwarfed and coupled. Old leaves develop distinct wavy margins, irregular necrotic interveinal areas and sometimes necrosis on veins (Figs. 16,17).

Fig.16. (A) Zinc deficiency in French bean: Plants with short internodes, interveinal chlorosis of old leaves and veins appear green, effects initiate from leaf base and margins.

Fig.17. (B) Zinc deficiency in French bean (close up): Affected old leaves with irregular interveinal chlorosis and necrotic patches, veins clear.

In cabbage, the young and middle leaves are distorted, wavy margins form cup like structure, the mature middle leaves sometimes are without curved margins. In certain cases the affected leaves show bronzing.

3.1.11. Molybdenum

Molybdenum is intimately associated with nitrogen metabolism. But its deficiency is known to result in disorganization of chloroplasts. It acts as a metal component of nitrogenase and nitrate reductase (Mengel and Kirkby 1987). Deficiency of molybdenum leads to the accumulation of nitrate as a result total proteins are disturbed. In addition to all these, molybdenum is known to be associated with alanine amino transferase (Agarwala *et al*. 1978) whose activity is lowered in low molybdenum. This in turn affects protein synthesis. Several iron enzymes are affected by low molybdenum. It is also known to have a role in the nucleic acids as in its deficiency the content of these acids are lowered. Role of Mo has also been established in reproductive physiology of higher plants where the quality of produce is also affected due to deficiency of the nutrient.

Molybdenum Deficiency

Molybdenum deficiency effects usually appear on those plants, which are grown on acidic soils. The deficiency symptoms of low Mo are discernible very late, almost on mature plants. In its deficiency growth of plants is stunted and lamina of old and middle leaves are reduced (Fig.18). In cauliflower and other Brassicas 'whiptail' like symptoms occur when the concentration of Mo is less than 0.2

Fig.18.   Molybdenum deficiency in spinach: (L) normal plant, (R) deficient plant. Growth and leaf size of deficient plants reduced, pale green foliage, scorching and downward rolling of leaf margins, old leaved bend down.

ppm on dry weight basis. In 'whiptail', lamina of new and middle leaves are distorted and puckered, the development of lamina remains incomplete and as a result, there is complete loss of lamina and the leaf is represented by midrib only. Growing point becomes blind. Young brassicas show cupping and interveinal necrosis of young and middle leaves.

In onion and related crop plants, the middle and old leaves give a wilted appearance due to death of tips hang down whereas the lower half remains green (Fig.19). Usually the deficiency symptoms are visible quite late at the time of appearance or emergence of inflorescence / flower. The flowers are usually abortive, under-developed and immature resulting in reduced yield.

Fig.19.   Molybdenum deficiency in brinjal: Middle and old leaves yellow, developed brown specks on chlorotic margins, leaf margins rolled downward and affected leaves hanged down.

3.1.12. Boron

The physiological role of boron in plants is in maintaining the structural integrity of cell membrane. In boron deficiency cell enlargement and division are retarded. It plays an important role in differentiation and maturation of plant cells. Boron suppresses apical meristem, in others, premature differentiation of mature tissues occurs. Boron is known to regulate water relations in plants. Deficiency of boron results in a higher rate of O_2 uptake and decreases respiratory phosphorylation and it is also involved in carbohydrate metabolism by playing a key role in sugar translocation (Dugger 1983). In another view, in boron deficient plants cellular activities of growing points of both tops and roots are reduced due to accumulation of sugars (Haas and Klotz 1931, Scripture and McHargue 1943, 1945, Milosavlgevic and Popovic 1970, Agarwala *et al*. 1978, Chatterjee *et al*. 1990, Neales 1959, Esteban *et al*. 1985). Accumulation of phenols (Steinberg 1955,

Grinkevich *et al*. 1970, Krupnikova and Smirnov 1981) and disturbances in growth regulations is another important feature of boron deficiency (Dyar and Webb 1961 and Hirsch and Torrey 1980). It is known to influence the nucleic acid metabolism in plants (Dugger 1983). Boron influences activity of many enzymes (Dutta and Mcllarth 1964, Buzover 1951, Carpena *et al*. 1978, Bonilla *et al*. 1980 and Agarwala *et al*. 1981). The development of reproductive parts of plants is also dependent on presence of boron. Boron plays an important role at all stages of development of both male and female reproductive parts and also at the level of flower formation (Gauch and Dugger 1954, Syworotkin 1958, Hewitt 1963 , 1983).

Boron Deficiency

In boron deficiency, the depression in growth of plants is apparent early almost with the initiation of visible effects. Boron deficiency usually affects young growth. The apical part of the young leaves of plants develop chlorosis which later spread to the entire lamina. The severely chlorotic lamina of the young emerging leaves collapse. The chlorotic as well as non-chlorotic leaves of boron deficient plants are thick and curl downward. In persistent boron deficiency the thick leaves crack from edges due to brittleness (Fig.20,21). The cracks are also present on petioles and pedicels of flowers (*e.g.* cauliflower), stem and midrib, which appear corky and sometimes roots are also split. In acute deficiency, death of growing point and distortion and blackening of new leaves with consequence loss of apical dominance and outgrowth of side shoots. Hollow stems and interveinal chlorosis are common symptoms of Brassicas. In boron deficient cauliflower 'Brown Heart'
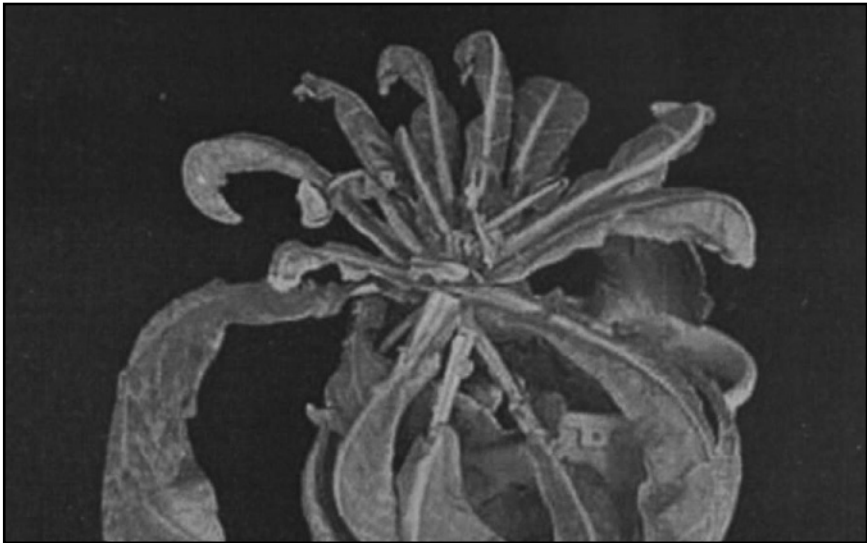
Fig.20. Acute Boron deficiency in cauliflower, plants deshaped, greater loss of leaf lamina, leaves not compact, spreading like rosette, leaves distorted, hook like lamina, dry from apex, appear thick, rugged and scorched, incomplete curd formation.

Fig.21. Boron deficiency in cabbage: Plant distorted, reduction in lamina, leaves thick from spatula like structure, not fully opened leaves, The young leaves rigid with torn margins, puckering and blackening of growing point.

and corkiness are specific symptoms. Prolonged deficiency causes death of growing point as well as in some cases black necrosis of young growth also occur (Fig.22).

Fig.22. Boron deficiency in tomato: Growth of plants, seized, internodes shortened, leaves thick, brittle, chlorotic, curled inward, leaf apex and margins scorched and dry, death of growing point.

The more susceptible vegetable are carrot, lettuce, radish, spinach, tomato, onion, sweet potato, etc.

Less susceptible are beans, cucumber, pea, potato, *etc.*

4. Experiments Under Controlled Conditions and Field Trials

Pot culture/ sand/ water culture and field trials have contributed tremendously towards our knowledge of manuring and fertilization of horticultural crops. Experiments under controlled conditions provide basic information which is combined with the data obtained from the field experiments to formulate tentative fertilizer recommendations for a particular fruit crop.

4.1. Soil Analysis

The supply of plant nutrients often control the chemical properties of soil. Soil analysis is an important method for gaining information regarding the mineral nutrition of plants. The soil analysis provides data on the total and available plant nutrients which are useful in formulating a fertility programme. To be more precise, data on soil pH, cation exchange capacity, texture, calcium carbonate, organic matter and total soluble salts should be collected and included before programming.

In recent times soil testing is becoming popular to access the fertility status with regard to both, macro and micronutrients; these tests are helpful in generating basic information on the status of soils (Table 3).

Assessment of soil fertility status involves an estimation of its available nutrient status. This phenomenon is referred to as soil testing and is used to optimize rate of fertilizer application. Nowadays incidences of micronutrient deficiencies in soils are increasing and several suitable tests for diagnosis and assessment of such deficiencies are employed for delineation of soil fertility, for making practical recommendations and for monitoring the nutrient status of soils (Table 4).

TABLE 3

Range of nutrient content commonly found in soils

Nutrient	Normal range	
	Percent	ppm
Nitrogen	0.02 - 0.50	200 - 5000
Phosphorus	0.01 – 0.20	100 - 2000
Potassium	0.17 – 3.30	1700 - 33000
Calcium	0.07 – 3.60	700 - 36000
Magnesium	0.12 – 1.50	1200 - 15000
Sulphur	0.01 – 0.20	100 - 2000
Iron	0.50 – 5.00	5000 – 50000
Manganese	0.02 – 1.00	200 – 10000
Copper	0.0005 – 0.015	5 – 150
Zinc	0.001 – 0.025	10 – 250
Molybdenum	0.00002 – 0.0005	0.2 – 5
Boron	0.0005 – 0.015	5 – 150

TABLE 4

Common soil tests and critical levels of nutrients in soil .

Element	Soil test method	Critical level in soil
Sulphur	0.15% $CaCl_2$	8-30 %
Calcium	Amm. acetate	< 0.25% of CEC
Magnesium	Amm. acetate	< 4% of CEC
Zinc	DTPA	0.6 ppm
Copper	DTPA	0.2 ppm
Iron	DTPA	4.5 ppm
Manganese	DTPA	2.0 ppm
Boron	Hot water	0.5 ppm
Molybdenum	Amm. Oxalate	0.2 ppm

The soil samples should be taken in a zigzag pattern at a depth of 0-15 cm. A representative composite soil sample comprising of 8-20 sub-samples from a uniform field should be consider. (Jones 1988, Sabbe and Marx 1987). For sampling auger or spade is quite satisfactory. For deep-rooted crop like horticultural crops, sampling

depth of 0-45 cm is desired. Samples from different depths or layers should be taken and analysed by the method described by Jackson (1958).

Soil tests are based on Viet's approach, which describes the amount of nutrient in a definite chemical form, *viz* (a) Water soluble (b) Exchangeable (c) Chelated or complexed (d) Secondary clay minerals or oxides and (e) Primary minerals/ The first three pools are thought to be important in supplying micronutrients for the plant during a growing season. The available micronutrients, therefore, do not reflect their total content in soils.

4.2. Determination of Macronutrients

The available concentration of nitrogen, phosphorus, potassium and sulphur are extracted in 0.15 % $CaCl_2$ and estimated according to the method of Subbiah and Asija (1956), Olsen (1958), Olsen *et. al.,* (1954), Jackson (1958) and William and Steinbergs (1959) respectively. Calcium and magnesium are extracted by ammonium acetate and determined either by flame photometer or calorimetrically.

Determination of Available Zinc, Copper, Manganese and Iron
Since long numerous studies have been conducted to find out a suitable extractant for simultaneous extraction of available Zn, Cu, Mn and Fe in test soils. Lindsay and Norwell (1978) developed a method using DTPA (Diethylene Triamine Penta Acid), which is suitable for identifying soils into deficient and non-deficient groups. This is a universally accepted method for analyzing available Fe, Mn, Cu and Zn in soils and estimated by Atomic Absorption Spectrophotometer. Available Molybdenum is extracted by Grigg's method (1953) and estimated by complexing molybdenum with the dithiol. Available Boron is extracted in hot water (Berger and Truog 1944) and analyzed by Wolf's method (1974) using Azomethane-H.

5.   Plant Analysis

A diagnosis based on symptoms, and confirmed by chemical analysis is the most reliable method of diagnosing nutrient disorders.

The plant analysis is dependent and based on certain principles as has been proposed by Aldrich (1967). These are:

· To identify or diagnose visible symptoms
· To identify hidden hunger
· To identify areas of incipient deficiencies.
· To indicate whether applied nutrients entered the plant or not.
· To indicate interactions or antagonisms among nutrients.
· To aid the understanding of internal plant functioning.
· Analysis of plant materials provides an idea of the nutrient concentration and (when multiplied by dry matter) of the total uptake.

The adaptation for leaf (tissue) analysis in perennial horticultural crops has proved its superiority over other diagnostic methods.

5.1. Sampling and Sample Preparation

For plant analysis to be more meaningful, collection of particular plant part at the right stage of growth as pre-technical specifications is very important (Table 5). It would be wrong and wasteful to just pluck any leaf or branch from a growing plant at any time and send to laboratory for analysis.

TABLE 5

Plant parts to be sampled and growth stage of crop for tissue analysis

Crop	Plant parts to be sampled	Stage of growth
Potato	4th to 6th leaf from growing tip	30-40 days after planting
Tomato	4th to 6th leaf from growing tip	Early bloom
Chilli	Young mature leaves	Early fruit set
Cauliflower	Young mature outside leaves	Button stage of curd
Cabbage	First mature leaf from central whorl	Prior to heading
Beans	2-3 fully developed leaves at top of the plant	Initial flowering
Root crops	Young mature leaves from central rings	Prior to root enlargement
Bulb crops	Young mature leaves from center	Prior to bulbing
Leaf greens	Youngest mature leaf	Mid growth

· Based on Knott's Handbook for vegetable crops, 1960, John Wiley and Sons, New York. Donahve,R.L., Miler, R.W. and Shicidune, J.C. 1990, Soil : An introduction to soil and plant growth. Prtntee-Hal of India Pvt. Ltd., New Delhi and Diagnosis and Improvement of Saline and Alkali Soils: Agriculture Hand Book 1954, No. 60 USDA. Oxford, IBH Publishing Co.

It is widely accepted that the greatest error in plant testing arises during sampling and that errors associated with sample preparation and analysis are usually less significant by comparison with other associated factors.

Leaf analysis can be misleading sometimes especially when phloem-immobile elements are dealt with, *e.g.* Ca and/or Cu as deficiency and adequacy may exist simultaneously in different parts of the same plant (Loneragan *et al*. 1976). In Ca/Cu deficiency - old leaves usually contain sufficient Ca / Cu in young leaves, the range of element concentration is very low (deficient range).

The basic principal behind this technique is that the nutrient concentration of plants is related to the amount of nutrient element available in soil. Leaf samples for analysis should be selected on the basis of physiological age *i.e.* development stage (Table 6). It is also important that the samples must be free from diseases, insect damage and physical or chemical injury. Leaf near the fruit should not be sampled as the nutrients that might have contained by the leaf are often translocated to the fruits. The form of nutrient accumulated within a plant is often influenced by the supply of that nutrient.

Nicholas (1957) in one of his experiments observed that chemical test for leaf analysis is the only certain way of differentiating between pathogenic and non-pathogenic (nutrient disorder) diseases.

TABLE 6

Sufficient and critical values of nutrients in leaves of vegetable crops

Nutrients	Sufficient level (ppm)	Critical level (ppm)
Iron	50 – 250	50 – 80
Manganese	30 – 200	30 – 50
Copper	8 – 20	4 – 8
Zinc	30 – 100	20 – 30
Boron	30 – 80	20 – 30
Molybdenum	0.5 – 5.0	0.2 – 0.5

5.2. Critical Concentration

To assess nutrient status of plants the concept of critical nutrient concentration is followed among several methods used for plant analysis. The definitions are implied in most of the modern diagnostic criteria / law that have been developed.

The concentration of the nutrient that denotes severe deficiency is that corresponds to 50% decrease in yield due to deficient supply of the nutrient is called as 'severe deficiency' (CSD).

The concentration of the nutrient that corresponds to 10% decrease in yield due to deficient supply of the nutrient denotes 'threshold of deficiency' (CTD).

The concentration of the nutrient corresponding to 10% decreased in yield owing to excess supply of the nutrient denotes 'threshold of toxicity' (CTT).

The concentration of nutrient corresponding to 50% decrease in yield due to toxic supply of the nutrient denotes 'severe toxicity' (CST).

It has been suggested that the critical concentration is not a single value but a narrow range of nutrient concentrations, above which the plant is supplied with high / ample amount of nutrient and below which the plant is deficient (Ulrich 1952). Such a range would, therefore, cover the different critical values derived by strict application various definitions (Table 7a, 7b and 8).

The nutrient levels in plant tissues are influenced by several physical, environmental and biological factors and these can be supportive to understand the derivations from the critical nutrient concentrations in a range rather than as a single value. Generally the ranges of critical concentrations are not considered and used by many workers.

Therefore a critical concentration of any nutrient is a range to provide estimates of the error involved in its derivation (Smith 1986). Different computer based non-linear regression models have now been worked out which also accommodate objective derivation of critical nutrient concentration (Griffiths and Miller 1973, Smith and Dolby 1977, Johansen 1978). Loneragan (1968) introduced a hypothesis of a functional nutrient requirement which was further modified as the minimal concentration of nutrient within the organism which can sustain its metabolic function at a rate which does not limit growth.

TABLE 7(a)

Critical concentration of macronutrients in vegetable crops.

Crop	Element	Concentration (%) denoting		
		Low	Moderate	High
Pea	N	1.8 – 1.9	2.0 – 3.5	> 3.5
	P	0.20 – 0.29	0.3 – 0.8	> 0.8
	K	1.8 – 1.9	2.0 – 3.5	> 3.5
	Ca	0.22 – 0.29	0.3 – 0.7	> 0.7
	Mg	0.22 – 0.29	0.3 – 0.7	> 0.7
	S	—	—	—
Cauliflower	N	2.80 - 3.29	3.30 – 4.50	> 4.50
	P	0.28 – 0.32	0.33 – 0.80	> 0.80
	K	2.0 – 2.59	2.60 – 4.20	> 4.20
	Ca	1.50 – 1.99	2.00 – 3.50	> 3.5
	Mg	0.22 – 0.26	0.27 – 0.50	> 0.5
	S	0.24 – 0.25	0.26 – 0.30	> 0.3
Cucumber	N	3.80 – 4.49	4.50 – 6.0	> 6.0
	P	0.28 – 0.34	0.34 – 1.25	> 1.25
	K	3.20 – 3.89	3.90 – 5.0	> 5.0
	Ca	0.90 – 1.39	1.40 – 3.5	> 3.5
	Mg	0.22 – 0.29	0.31 – 1.0	> 1.0
	S	0.25 – 0.39	0.40 – 0.7	> 0.7
Spinach	N	3.50 – 3.90	4.0 – 6.0	> 6.0
	P	0.25 – 0.29	0.3 – 0.6	> 0.6
	K	4.0 – 4.99	5.0 – 8.0	> 8.0
	Ca	0.50 – 0.69	0.7 – 1.2	> 1.2
	Mg	0.40 – 0.59	0.6 – 1.0	> 1.0
	S	0.19 – 0.23	0.24 – 0.26	> 0.26
Beans	N	4.24 – 4.99	5.0 – 6.0	> 6.0
	P	0.25 – 0.34	0.35 – 0.75	> 0.75
	K	2.0 – 2.24	2.25 – 4.0	> 4.0
	Ca	1.0 – 1.49	1.50 – 2.50	> 2.5
	Mg	0.25 – 0.29	0.30 – 1.0	> 1.0
	S	0.26 – 0.28	0.28 – 0.30	> 0.3
Brinjal	N	3.50 – 3.99	4.0 – 6.0	> 6.0
	P	0.25 – 0.29	0.30 – 1.2	> 1.2
	K	3.0 – 3.49	3.5 – 5.0	> 5.0
	Ca	0.80 – 0.99	1.0 – 2.5	> 2.5
	Mg	0.25 – 0.29	0.3 – 1.0	> 1.0
	S	0.21 – 0.24	0.25 – 0.26	> 0.26

TABLE 7(b)

Critical concentration of macronutrients in vegetable crops

Crop	Element	Concentration (%) denoting		
		Low	Moderate	High
Carrot	N	1.80 – 2.09	2.10 – 3.5	> 3.5
	P	0.17 – 2.09	2.10 – 3.5	> 3.5
	K	2.50 – 2.79	2.80 – 4.0	> 4.0
	Ca	0.80 – 1.39	1.40 – 3.0	> 3.0
	Mg	0.25 – 0.29	0.30 – 0.5	> 0.5
	S	0.19 – 0.21	0.22 – 0.30	> 0.3
Radish	N	2.80 – 2.99	3.0 – 6.0	> 6.0
	P	0.25 – 0.29	0.3 – 0.7	> 0.7
	K	3.50 – 3.99	4.0 – 7.5	> 7.5
	Ca	2.0 – 2.99	3.0 – 4.5	> 4.5
	Mg	0.30 – 0.49	0.5 – 1.2	> 1.2
	S	0.16 – 0.20	0.21 – 0.25	> 0.25
Onion	N	4.50 – 4.90	5.0 – 6.0	> 6.0
	P	0.25 – 0.34	0.35 – 0.5	> 0.5
	K	3.50 – 3.99	4.0 – 5.5	> 5.5
	Ca	0.80 – 0.99	1.0 – 2.0	> 2.0
	Mg	0.22 – 0.24	0.25 – 0.40	> 0.4
	S	0.30 – 0.49	0.50 – 1.0	> 1.0
Garlic	N	3.0 – 3.89	3.90 – 4.8	> 4.8
	P	0.25 – 0.29	0.30 – 0.6	> 0.6
	K	3.0 – 3.89	3.90 – 4.8	> 4.8
	Ca	0.10 – 0.14	0.15 – 0.25	> 0.25
	Mg	0.10 – 0.14	0.15 – 0.25	> 0.25
	S			
Turnip	N	3.0 – 3.49	3.5 – 5.0	> 5.0
	P	0.28 – 0.32	0.33 – 0.6	> 0.6
	K	3.0 – 3.49	3.5 – 5.0	> 5.0
	Ca	0.25 – 0.29	0.3 – 1.0	> 1.0
	Mg	0.25 – 0.29	0.3 – 1.0	> 1.0
	S			
Tomato	N	1.05 – 2.89	2.9 – 5.0	> 5.0
	P	0.20 – 0.24	0.25 – 0.75	> 0.75
	K	1.05 – 2.89	2.9 – 5.0	> 5.0
	Ca	0.25 – 0.39	0.4 – 0.6	> 0.6
	Mg	0.25 – 0.39	0.4 – 0.6	> 0.6
	S	0.25 – 0.39	0.4 – 1.2	> 1.2
Lettuce	N	6.5 – 7.4	7.5 – 9.0	> 9.0
	P	0.3 – 0.4	0.5 – 1.0	> 1.0
	K	0.3 – 0.4	0.5 – 0.8	> 0.8
	Ca	0.3 – 0.4	0.5 – 0.8	> 0.8
	Mg	0.3 – 0.4	0.5 – 0.8	> 0.8
	S			

TABLE 8
Critical concentration of micronutrients in vegetable crops.

Crop	Element	Plant part	Concentration (ppm) denoting				
			Severe deficiency	Threshold of deficiency	Sufficiency/ adequacy	Threshold of toxicity	Severe toxicity
Pea	Mn	M.L.	< 12	20	22 – 80	100	
	Cu	Y.L.	< 4	10	11 – 14	14	> 16
	Zn	M.L.	< 12	20	22 – 80	80	> 200
	B	L.	< 3	10	11 – 50	50	
Cowpea	Zn	L.	< 20	45	50 – 150	150	
	B	Y.L.	> 9	12	13 – 75	75	
	Fe	Tops	< 70		> 100		
Cabbage	Cu	L.		5	5.2		
	Zn	L.	< 10		10 – 200		
	Fe	L.	< 50		50	60 – 200	
	B	L.	< 20		30 – 60		
	Mo	L.	< 0.1	0.2	0.3 – 0.5		
Tomato	Fe	L.					
	Mn	L.	< 25				
	Cu	Y.L.		25 – 50	50 – 500		
	Zn	Y.L.	< 20	20 – 30	5.15		
	Mo	Y.L.	0.13	0.68	30 – 200		
	B	Y.L.	< 12		51 – 88	> 172	
Carrot	Fe	Y.L.			120 – 350		
	Mn	Y.L.			190 – 350		
	Cu	Y.L.	< 5		10 – 25	> 332	
	Zn	Y.L.		18	20 – 50		
	B	Y.L.		20	29 – 35		
Cucumber	Cu	Y.L.	< 8		7 – 10	> 10	
	Zn	Y.L.			20 – 40		
	B	Y.L.	< 20		40 – 120	> 300	
Potato	Fe	Y.L.			70 – 150		
	Mn	Y.L.	< 20	20 – 40	40 – 300	> 1000	
	Cu	Y.L.	< 3	3.5	6 – 20		
	Zn	M.L.	< 10	10 – 15	15 – 30		
	Mo	M.L.		0.1	0.1 – 1.5		
	B	Y.L.	< 10	10 – 20	20 – 50		
Spinach	Fe	Y.L.			220 – 245		
	Mn	Stem	12		31		
	Cu	Y.L.			45 – 65		
	Zn	Y.L.			50 – 75		
	Mo	Y.L.	0.1		1.61		
	B	Y.L.			42 – 63		

L – Leaves;   Y.L.- Young leaves;   M.L. – Middle leaves; O.L. - Old Leaves

The relationship between yield and nutrient concentration is revealed by a well defined curve which is required for derivation of a critical nutrient concentration. To obtain this, it is necessary to grow plants either in a water culture or sand culture or through field experiments (Fig.23).
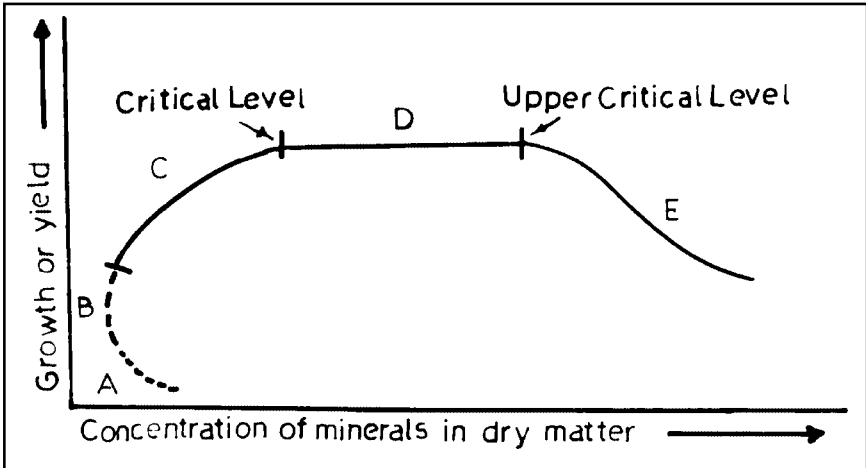
Fig.23. Relation of mineral composition to growth or yield. (Hewitt 1983).

A&B	-	Severe deficiency	C	-	Moderate Dificiency
D	-	Luxury range	E	-	Toxic range

It is also essential to identify that the two genotypes having the same critical concentration of a nutrient in a specific plant part may have very different external requirements for that element, therefore, the inorganic fertilizer recommendations may also vary for the different genotypes of the same plant species because in several instances the genotypes differ in their ability for uptake, absorption and translocation.

6.   Amelioration of Nutrient Deficiencies

6.1. Macronutrients

In natural ecosystems, the minerals absorbed by the crops return to the soil after organic matter decomposition and soil fertility is more

or less maintained through nutrient cycling. In cultivated ecosystems like vegetable cultivation however, all harvested biomass (product and plant residue) withdrawn from the field contains nutrients that no longer return to the soil. Hence, maintenance of soil fertility and crop yield should depend on counter balancing of fertilizer inputs.

Chemical fertilizers are inorganic or synthetic materials of concentrated nature. They contain one or more plant nutrients in easily soluble and quickly forms.

6.1.1. Nitrogen

Generally nitrogen is the limiting element for plant growth and the vegetable crops require more of nitrogen than any other nutrient. Nitrogen is highly mobile and easily lost from the soil. In cultivable land, crops can utilize only 50-60% of the available nitrogen.

Nitrogen use efficiency in vegetable crops can be increased by adding the following measures :

i.   Split application of nitrogen fertilizers at the time of peak requirements of the crop which decreases nitrate leaching and thereby increases nitrogen use efficiency.
ii.   Plants can take 30-35% more nitrogen when applied deep in soil rather than applying on surface.
iii.   Optimum soil moisture increases nitrogen use efficiency
iv.   Nitrogenous fertilizers should not be applied in large quantities near the root zone because of the danger of salt damage.
v.   When adequate P and K fertilizers are applied along with nitrogen to the soil; the use of nitrogen appears to be most efficient.

Generally half of the required nitrogen dose fertilizers are given as basal dose at the time of final land preparation and rest in splits, but in leguminous vegetable crops, entire nitrogen fertilizer is applied as basal dose.

6.1.2. Phosphorus

Phosphorus use efficiency can be increased by adopting the following :

i.   Maintenance of soil pH at 6.5 – 7.0
ii.   Phosphatic fertilizers should be applied as basal dose near the active root zone of the plant.
iii.   Maintenance of organic matter in soil increases P availability to the plants.
iv.   Late application of phosphorus is not effective for plant growth.
v.   In cold weather higher phosphorus doses should be applied because the absorption of P is less in this weather.
vi.   Adequate moisture in soil and combined application of N and P fertilizers increase phosphorus availability to the plants.
vii.   Rock phosphate or basic slag should be applied 2-4 weeks before sowing or transplanting

6.1.3. Potassium
i.   Maintenance of soil pH at 6 – 7 by liming which reduces leaching.
ii.   Split application of potassic fertilizers in loose soils of high rainfall areas.

6.1.4. Sulphur
i.   Maintenance of soil pH to 6 – 7 by liming.
ii.   Application of organic matter to the soil.
iii. In acute deficiency, elemental sulphur may be applied before sowing / transplanting. Elemental sulphur may produce soil acidity therefore it is desirable to apply calcium in the form of lime at the time of sulphur application to the soil.

6.2. Micronutrients

The main reasons of accelerated exhaustion of available micronutrients in the soil are as follows.
i.   Growing of high yielding cultivars, which demand more micronutrients.
ii.   Application of more N, P and K fertilizers as they decrease the availability of micronutrients.
iii. Inadequate application of organic matter to the soil
iv.   Intensive cropping, which creates deficiency.
v.   Leading loss of micronutrients particularly in light-textured soils of high rainfall areas.
vi.   Application of compound fertilizers is more effective than application of single fertilizer because they may add more micronutrients.
vii. Maximum application of phosphatic fertilizers decreases the availability of zinc, whereas application of sulphate compounds increases the availability of zinc.
viii. Moisture stress conditions decrease the availability of Fe and B while excess moisture conditions causes that of zinc. However excess moisture condition are not normally met with vegetable cultivation. Micronutrient deficiency can be categorized into two types *i.e.* primary and secondary deficiency and thereby managed accordingly.

6.2.1. Primary Deficiency
i.   This refers to the low content of the nutrients which depend on the soil type and other agroclimatic conditions.
ii.   Sandy and calcareous soils contain less micronutrient elements, whereas clay loam and loam soils contain more micronutrients.
iii. Loose textured soil are low in organic matter.
iv.   Soils of high rainfall areas are deficient in micronutrients.
v.   Application of micronutrients should be applied in deficient conditions only, otherwise increase in micronutrient levels in soil may cause phytotoxicity.

6.2.2. Secondary Deficiency

i.   Total micronutrient contents in the soil may be ample, but their availability is restricted due to other factors like soil pH, interaction of nutrients etc.

ii. Availability of Zn, Mn, Fe, Cu and B is often reduced in soils exhibiting alkaline reactions, whereas Mo is unavailable in acidic soils.

iii. Secondary deficiencies may be corrected by proper soil management like liming, application of organic matter to the soil *etc*.

Under Indian condition the vegetable crops show favourable response on application of micronutrients. Generally micronutrients are applied in four different ways to the soil. These are (Table 9) :

TABLE 9

Application of micronutrients

Nutrients	Soil application of nutrient (kg/ha)	Foliar application (concentration)
Iron	0.5 – 10.0	0.4% ferrous sulphate + 2% lime
Manganese	5.0 – 12.0	0.4-0.6% manganese sulphate + 0.2-0.3% lime
Copper	3.0 – 8.0	0.1-0.2% copper sulphate + 0.5% lime
Zinc	0.5 – 8.0	0.2-0.6% zinc sulphate + 0.1-0.3% lime
Molybdenum	0.05 – 1.0	0.05% sodium or ammonium molybdate
Boron	0.5 – 5.0	0.5-0.6% borax

7. Assessment of Nutrient Status of Plants by Biochemical Parameters

Brown and Hendrick (1952) proposed a hypothesis by which the nutrient status of plants can be assessed on the basis of enzyme activity. The hypothesis says that "if an element is limiting in the nutrition of plant, the deficiency will be evident in changed enzyme activity, as the enzyme requires that particular element for its function" *e.g.*, the activity of ascorbic acid oxidase is markedly reduced by limited copper supply or catalase is reduced when iron supply is low or activity of peroxidase is increased markedly when manganese is low and decreased significantly when iron is limited, thus Bar-Akiva (1961) suggested peroxidase activity to be as an indicator of Mn deficiency of plants. Kessler (1957) observed that the activity of ribonuclease can be an index of zinc availability not only for field crops but also for

fruit trees which was supported by Dwivedi and Randhawa (1974). To distinguish zinc or copper deficiency, the changes in the activity of carbonic anhydrase or ascorbic acid oxidase is used and has been suggested to be metabolic indicators for zinc and copper status. The activity of nitrate reductase can also be used as an index for molybdenum status of plants (Chatterjee *et al*., 1985).

In plants the decrease or increase in enzyme activity may cause either the accumulation or disappearance of certain metabolic products, which can also be helpful in diagnosing any nutrient disorder for example, the variation in the concentration of plastocyanin is one of the most reliable indicator of copper status (Plesnicar and Bendall, 1971) of plants.

These observations and some of our results on biochemical parameters support the evaluation of nutrient stress both under controlled conditions and in fields along with specific visible symptoms or sometimes biochemical parameters are alone helpful when stresses are latent.

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6

Major Fungal and Bacterial Diseases of Potato and their Management

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ABSTRACT : Potato is an important crop which holds promise for food to millions of people especially in developing countries. Full potential of the crop can be realized only if diseases that affect the crop are kept under control. Major fungal diseases such as late blight, early blight, black scurf, fusarial wilt/dry rot, wart, powdery scab, charcoal rot and major bacterial diseases like soft rot, common scab, bacterial wilt and brown rot cause considerable loss to potato production in field and otherwise. Diseases such as late blight, early blight, fusarial wilt and black leg primarily affect the crop/foliage where as diseases such as black scurf, wart, powdery scab and common scab disfigure the tubers and reduce their market value. Some tuber diseases such as dry rots appear mostly in storage while others such as soft rot affect potato tubers at every stage *i.e.* in field, storage and in the transit and may cause substantial loss under certain conditions. Major fungal and bacterial diseases affecting potato crop are reviewed here with respect to their identifiation, symptoms on potato plants or tubers, nature of the pathogen involved, epidemiology, control measures *etc*.

1.   Introduction

Potato is a major food crop after wheat, rice and maize. Over next three decades when the world population is expected to grow by around 100 million a year and put further pressure on land, water and other resources, farmers in developing countries have to double their output to feed the growing numbers (Zandstra, 2000). In that scenario, potato holds promise for food to millions of people especially in developing countries. Full potential of the crop can be realized only if diseases and pests are kept under control. Potato crop can be affected by approximately 160 diseases and disorders of which 50 are caused by fungi, 10 by bacteria, 40 by viruses and others by non parasitic, or due to unknown causes. Diseases may affect potato at any stage of

crop growth or even during storage. They may affect foliage, tubers or both. Environment favouring pathogens can ruin the crop. The fallouts of historical potato famine in Europe particularly in Ireland caused by late blight have been well documented (Woodham- Smith, 1962). Tuber diseases like common scab, black scurf, dry rots, soft rot may not destroy the crop but can greatly reduce quality and marketability of the crop. With the introduction of resistant varieties and improved cultural practices, the disease scenario may change from time to time which require periodic surveillance (Khurana, 1998; Khurana *et al*., 1998). Diseases may also be affected by any change in environment such as global warming (Kankoranta, 1996). Reviews on fungal and bacterial diseases in Indian context are available (Khurana, *et al*. 1999; Shekhawat *et al*. 1999; Singh and Hegde, 1999; Verma and Sharma, 1999). The present review incorporates up-to-date information on developments that have taken place both in India and elsewhere. Information is arranged under headings : symptoms, pathogen, epidemiology and control for each of important fungal and bacterial diseases. The information can be used in better management of the crop.

2.   Fungal Diseases

Major fungal diseases, which affect potato crop are late blight, early blight, black scurf, dry rots, wart, powdery scab and charcoal rots. Brief description and control measures for each of these diseases is discussed.

2.1 Late Blight

Late blight is the most dreaded disease of potato world over. It cuts global potato production by around 15 %. Overall annual cost of late blight in developing countries alone is estimated at $ 3.25 billions (Mackin, 1998). A comprehensive survey conducted to estimate the impact of late blight on potato yield, storage losses and fungicide use in the United States revealed that the fungicides cost $ 77.1 million and loss to revenue was an additional $ 210.7 millions. Cost to manage

the disease average around $507 per ha which do not include non-fungicide control practices (Guenthner *et al*. 2001). Late blight even in the present times can raise fear of famine in vast area of Eastern Europe and Russia where million of people are still subsisting on potatoes (Mackin, 1998). Losses due to late blight in different countries have been reviewed by Cox and Large (1960). For past one and half century since Irish potato famine in 1944-45 the disease has occupied centre stage of plant pathological research world over and lot of work on this disease has been carried out both in temperate and tropical climate. The information on various aspects of late blight have been reviewed (Crosier, 1934; Black, 1952; Woodhouse, 1962; Deweille, 1964; Hori, 1964; Gallegly, 1978; Erwin *et al*., 1983; Neiderhauser,1986; CIP, 1989; Lucas *et al*., 1991; Ingram and Williams, 1991; Arora and Khanna, 1997; Singh and Bhattacharyya, 1998; Singh and Shekhawat, 1999).

2.1.1. Symptoms

Late blight appears first as water- soaked irregular pale green lesions mostly near tip and margins of leaves. These lesions rapidly grow into large brown to purplish black necrotic spots (Fig.1). During morning hours a white mildew, which consists of sporangia and spores of the pathogen, can be seen on lower surface of infected leaves especially around the edges of the necrotic lesions (Fig.2). Light to dark

Fig.1: Late blight symptoms on leaves

brown lesions appear on stems or petiole which elongate and encircle the stems. The affected stems or petiole become week at these locations and may collapse. Under disease favorable conditions entire crop gives blackened blighted appearance (Fig. 3) and may be killed with in a week. Tubers in soil become infected by rain borne sporangia from the diseased foliage. The infected tubers show irregular reddish brown to purplish slightly depressed areas which extend deep into internal

Fig.2:   Sporulation of *Phytophthora infestans* on leaves

Fig.3: A field affected by late blight

tissues of the tubers (Fig. 4). The infected tubers initially are dry, firm and hard but may be invaded by other pathogens mainly bacteria and develop soft rot.

Fig.4 : Late blight infection in potato tubers

2.1.2. The Pathogen

Late blight is caused by *Phytophthora infestans* (Mont.) de Bary. It belongs to order Peranosporale*s* of class Oomycetes. The fungus is characterized by lemon shaped detachable, papilliate sporangia produced on sympodially branched sporangiophores of indeterminate growth. The sporangiophores exhibit a characterized swelling at junction where sporangia are attached with the sporangiophores. Fungal development, nutrition, biochemistry, cell biology and population biology of *P. infestans* has been reviewed by Ingram and Williams (1991). Cytology, cytogenetics and genetics of *P. infestans* have been reviewed by Ehrlich and Ehrlich (1966). The fungus is heterothallic and requires two mating types A_1 and A_2 for sexual reproduction. Prior to 1984 the A_2 mating type was restricted to Mexico and Andean mountains the center of origin of cultivated potatoes. First report of A_2 mating type outside Mexico was from Switzerland (Hohl and Iselin, 1984). Subsequently it was reported from other countries as well (Malcolmson, 1985; Mosa *et al.*, 1989; Shaw *et al*. 1984; 1985; Fry *et al.*, 1989; Fry and Spielman 1991; Singh *et al.*, 1994). This is considered as second migration *P. infestans* outside Mexico (Fry *et al*,., 1999), the first being from Europe and America during the historical potato famine around the year 1845. The new strains of the pathogen are far more aggressive than the old population (Fry *et al.*, 1999). Turkensteen and Mulder (1999) reported that pathogen, during the last 20 years has, developed a shorter life cycle (by 30%), ability to cause more leaf spots, shorter infestation period (6 instead of 8h), tolerance to a greater temperature range (5 to 27⁰C instead of 10 to 25 ºC), forms stem lesions more

frequently, develops oospores and sporulation on tubers and is more inclined to develop resistance to fungicide metalaxyl. Population of *P. infestans* in most countries has changed dramatically and original A_1 have almost been displaced by more virulent A_2 strain (Peters *et al*. 1998). Occurrence of both A_1 and A_2 strains at the same location has opened up the possibility of development of thick walled oospores which could survive either extreme winter (Medina and Platt, 1999) or summers conditions. The oospores may act as another source of primary inoculum, in addition to the already known sources such as infected seed tubers; waste heaps, volunteer plants etc.

2.1.3.   Epidemiology

The pathogen over winters as mycelium in infected tubers in refuse piles and volunteer plants or over summer through cold stores (Pushkarnath and Paharia, 1963; Boyd, 1981). Such infected tubers serve as primary source of inoculum. Survival of pathogen in soil as oospores has added yet another dimension to the source of primary inoculum but its exact role and extent of contribution is not clear. Movement of pathogen from infected tubers to shoots could be indirectly through soil. The pathogen under favourable conditions may sporulate on surface of tuber, liberate zoospores in soil which move upward and infect the plant at soil level (Arora, 1996). The stem below ground resists the infection. Leaves touching ground gets infected first. Further spread of the pathogen takes place either by air or water borne sporangia. Sporangia are sensitive to desiccation and require free water for germination. Optimum temperature for development of zoospores in sporangia is 12 ºC. It may take only 30 minutes to produce zoospores at this temperature. The zoospores are disseminated by splashing rain drops and cause rapid development of the disease in field. Zoospores produce germ tubes and appresoria in presence of free water and penetrate the host tissue within two to two and half hour at 10 to 25 ºC. Once the penetration has occurred, subsequent development of the disease is most rapid at 17 to 25 ºC, optimum being 21 ºC when lesion with fresh sporangia appear within 3 to 5 days.

Tubers in soil get infected by contact with sporangia coming from infected haulms through rain water or under wet conditions at harvest. The infection can also occur during washing of tubers. Fairclough *et al*. (1997) determined that a single blighted potato (cv. Home Guard) tuber releases on an average 1.39mg of *P. infestans* mycelia and sporangia during simulated washing. Up to 100% tubers were infected when healthy tubers were washed in a suspension of *P. infestans* equivalent to 1.65ug / ml which can be provided by approximately one % of blighted tubers. Immature tubers were more prone to infection than the mature tubers.

Several models to forecast late blight have been developed (van Everdingen, 1926; Beamount, 1947; Bourke, 1955; Hyre, 1954; Wallin, 1962; Ulrich and Schrodter, 1966; Krause *et al*. 1975; Bruhn and Fry, 1981; Bhattacharya *et al*., 1982, Singh *et al*. 2000) but the most successful and widely used models were 'BLITECAST' developed by Krause *et al*.(1975) and SYMPHYT developed by Bruhn and Fry (1981). Computer aided decision support systems *viz*.' Negfry'

and 'Paso' and another called 'l@nteinfo' (www.planteinfo.dk) is available *via* the internet (Hensen *et al*., 2000). Similarly a potato late blight alert network consisting of 33 automated units has been in operation in New Brunswick, Canada since 1996 with satisfactory results (Cho and Bernard, 1999). International Potato Center has linked two disease forecasting models, Blitecast and Simcast to climate database in a geographical information system (GIS) to estimate global severity of potato late blight. Global zonation of estimated late blight severity was found similar from both forecast models, but Blitecast generally predicted lower number of sprays. Zone of high late blight severity are the tropical highlands, Western Europe, East Coast of Canada, Northern USA and South Eastern China. Major production zones with a low late blight severity include Western plains of India, where irrigated potato is produced in the cool dry season, North Central China and North- Western USA. Average number of sprays calculated for different countries using GIS database of potato production compared with estimated current fungicide use revealed that the estimated number of sprays in developing countries whether from Blitecast or Simcast, did not correlate with the observed number of sprays. It predicted optimum number of sprays much higher as compared with the actual number observed. On the basis of GIS database it was suggested that an increase access to host resistance and fungicides in developing countries could have a strong economic impact on potato production (Hijmans *et al*. 2000). Different methods and weather criteria may be required for forecasting potato blight for different regions. Based on local weather parameters a computerized forecast for late blight named as 'JHULSACAST" has been developed for western subtropical plains of India (Singh *et al*., 2001).

2.1.4. Management

Control of contaminated sources such as waste heaps, infected tubers, volunteer plants, disease in neighboring fields and regrowth after destruction of haulms can help in management of the disease (Turkensteen and Mulder, 1999). Choice of suitable cultivars, well aerated fields, pre- sprouting of tubers, early planting and use of resistant varieties are some of the measures against foliar blight while planting potatoes on large steep ridges, right time of mechanical weeding and harvesting, avoiding rapid shift of harvested tubers and long transports could minimize tuber blight (Meinck and Kolbe, 1999). In Switzerland, it has been estimated that onset of epidemic can be delayed by 3 to 6 weeks if all primary infection from early potato can be eliminated (Forrer *et al*., 2000). Increased application of nitrogen can lead to increase in disease severity and more fungicides or use of resistant varieties may be required to manage the disease. However, higher dose of phosphorus and potassium was found to give a positive response to yield in a late blight year (Roy *et al*., 2001).

Development of resistant cultivars has played an important role in the control of late blight. *Solanum demissum*, a hexaploid wild species, has extensively been used to confer resistance against *P. infestans* in the early twentieth century. Since the fungus is quite plastic and mutable, matching races against major R genes come up readily to overcome the resistance of the new cultivars. Field resistance

is polygenic and more durable. *Solanum bulbocastnum*, *S. microdontum*, *S. verrucosum* and *S. chacoense* have been used as a source of field resistance in breeding programs. However, major genes are still in use and new project 'RETONA' in USA aims to replicate in modern varieties, the multilineal control of late blight which has evolved naturally in *S. demmisum* population. These populations have survived for thousands of years where late blight occurs annually. It aims to incorporate combination of 16 resistant genes (R genes) identified so far, into commercial varieties (Niederhauser *et al.*, 1996).

Use of host density as a tool for management of late blight has also been viewed promising to control late blight. Tuber yield from both resistant and susceptible cultivars increase when grown together as mixture than the single genotype stands (Garrett and Mundt, 2000).

Spraying with an effective fungicide is a standard procedure for control of late blight. Bordeaux mixture, consisting of copper sulfate, hydrated lime and water, was a standard fungicide for many years. Subsequently the organic fungicides, especially carbamates which controlled both early and late blight and also were less toxic to potato replaced Bordeaux mixture. Metalaxyl – a phenylamide group of fungicides specific to oomycetes however, revolutionized late blight control (Bruck *et al.*, 1980). This fungicide not only prevent onset of late blight but also has potential to dry off the already formed lesions. Since it was most effective its use increased rapidly and this became one of the major fungicide used world over. But, within a very short span of time strains of *P. infestans* which do not respond to metalaxyl appeared (Dowley and Sullivan, 1981; Gisi and Cohen, 1996). Use of metalaxyl for control of late blight is so important that it forms backbone of the disease control program. Appearance of metalaxyl resistance in *P.infestans* could thus pose serious threat to manage late blight.

In India, resistance to metalaxyl in *P. infestans* wild population was first observed in Nilgiri hills of South India in 1989. Metalaxyl resistant strains appeared toward end of summer crop season and their frequency increased to 13 % in autumn season (Arora *et al.*, 1992a). The metalaxyl resistant strains were more aggressiveness than the sensitive strains as evident by their short incubation period in host, quick germination of sporangia to zoospores and ability to cause larger lesions (Arora, 1994). Metalaxyl in mixture with unrelated contact fungicide however, could retard development of resistance in the pathogen (Gangawane *et al.* 1993). Fungicide Resistance Action Committee (FRAC) of International Group of National Associations of Agrochemical Manufacturers set up country specific working groups for phenylamide fungicides. This has developed certain guidelines for management of resistance to metalaxyl that include using metalaxyl always in pre packed mixture with contact fungicide, restricting its use early in the season, avoiding curative use, sub-lethal doses or spray interval longer than recommended. Cymoxanil mixtures have been found effective for managing metalaxyl resistant strains (Samocha and Cohen, 1988). A synergism between cymoxanil and mancozeb has also been reported by Evenhuis *et al.* (1996).

Heavy dependence on fungicides could pose threat to environment and human population. Such questions as are potato fungicides used rationally?

(Bradshaw *et al*., 2000) or are excessive blight sprays detrimental to potato yield (Taylor *et al*., 2000) have been raised. A few commercial fungicides have also been reported to induce oospore formation and phenotypic changes in *P. infestans* (Groves and Ristaino, 2000). Scientific community is now devising ways and means to reduce heavy dependence on fungicides. Use of biocontrol agents is considered a safe option to the use of fungicides. Antagonism to *P.infestans* by some naturally occurring microorganisms such as *Trichoderma viride*, *Penicillium viridicatum*, *Chetomium brasilense* (Arora *et al*., 1992b); *Acremonium strictum*; *Myrothecium verrucaria*, *Penicillium aurantiogriseum* (Roy *et al*., 1991), *Epiccocum purpurascens*, *Stahcybotrys coccodes*, *Pseudomonas syringae*, *Fusarium graminearum* (Kim-Byuna Sup *et al*., 1996) and *Pythium ultimum* (Kuzuetsova *et al*., 1995) have been reported. Biocontrol agents have been found effective against late blight disease under controlled conditions such as laboratory and glasshouse but are less effective in field (Arora, 2000a). An integrated use of biocontrol agents with low dose of fungicides however, have shown some promise.

2.2. Early Blight

Early blight is a well known disease of potato and tomato. The disease appears earlier than late blight in USA hence the name early blight. However the name is misleading because the disease rarely attacks young growing plants and more often affects mature and old plants showing loss of vigor. Early blight occurs in Asia, Africa, Australia, Europe, North, Central and South America (Miller and Pollard, 1976) and may cause annual losses between 10 to 25 %. The disease is more severe under alternate dry and wet climate where the annual losses from this disease sometime may even exceed that caused by late blight.

2.2.1. Pathogen

Early blight is caused by *Alternaria solani* Sorauer (Ellis and Martin) belonging to Deuteromycetes. Other species of *Alternaria* which attack potato are *Alternaria alternata* (Fries) Keissler, and *A. consortialis* (Conner, 1967). *A. solani* has septate mycelium and bears conidia on erect and septate conidiophores. Conidia are obclavate, olive brown with tapering long filiform beak. The conidia are multicellular and possess 3 to14 cross septa and 0 to 18 longitudinal septa (Western, 1971). Cultural characters vary widely on potato dextrose agar medium. Colonies of the fungus are spreading, grey brown to black occasionally with yellow red pigment in the media. The mycelium sporulates sparingly on media, however, sporulation can be induced by mutilation of mycelium or exposing the culture to different light sources (Singh, 1967; Barksdale, 1969). Physiological races of the pathogen have been recognized (Thomas, 1943). Spores germinate in water within an hour at optimum temperature range between 24 to 30 ºC.

2.2.2. Symptoms

Numerous small, round, oval or angular, dark brown to black, dry and papery necrotic spots which have angular margins appear on leaves. These spots are generally limited by leaf veins (Fig 5). Concentric rings of raised and depressed tissue within the leaf spot gives it a 'bull's eye or target' appearance. Leaf tissues around the spots often become chlorotic and yellow suggesting involvement of toxins. On tubers, the lesions are dark, sunken, circular to irregular in shape, shallow and separated by healthy tissue by purplish- brown dry cork layer. Early blight lesions are less prone to secondary infections.
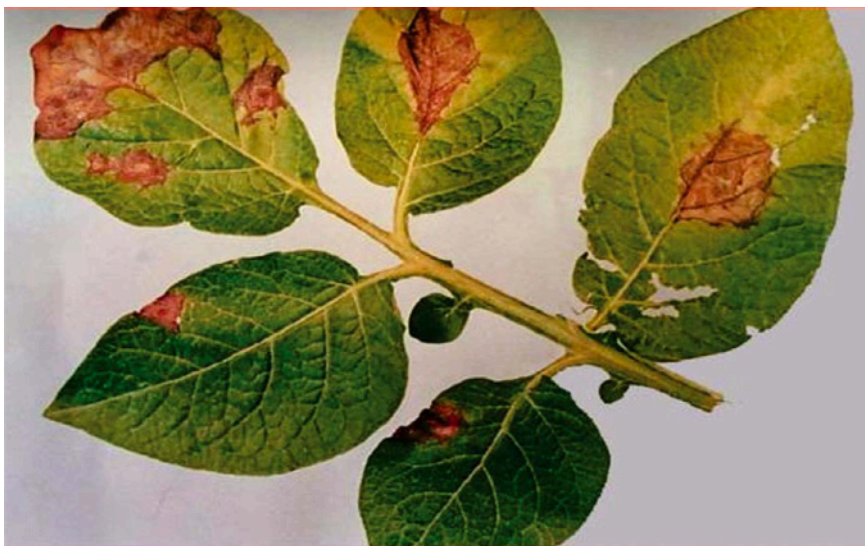
Fig. 5:   Early blight symptoms on leaves

2.2.3.   Epidemiology

A. *solani* survives in crop debris, soil, infected tubers or alternate hosts which act as primary source of inoculum. The disease is favoured by short rotation, continuous cropping of potatoes and tomato (Manzer and Merriam, 1974). Infection is favoured by warm temperature and alternating high relative humidity provided by heavy dew, light rains or irrigation (Dutt, 1979; Eastons *et al*., 1975; Harrison *et al*., 1965; Ohms and Fenwick 1961; Rotem and Reichert, 1964). Temperature in the range of 25 to 30 ºC is congenial for the disease (Barclay *et al*., 1973; Singh *et al*., 1987). Actively growing, properly fertilized young plants do not exhibit the disease. Wounded or immature tubers are prone to infection. A delay of 10 to 15 days between haulm destruction and harvest prevents infection in tubers. Late maturing cultivars are generally more resistant than the early varieties. Predisposition of plants to injury, poor nutrition or other stresses may favour the disease development.

2.2.4. Management

Removal and destruction of diseased haulms from infected fields reduces sources of primary inoculum for the next crop. Cultivation of solanaceous crops, being collateral hosts, near potato fields must be avoided. Applying recommended dose of fertilizers especially nitrogen ensures healthy and vigorous growth and less disease. Permitting tubers to mature in soil and avoiding bruises at harvest minimize tuber infection. Sprinkler irrigation favours disease and should not be used more often than necessary. Crop sprayed with one percent urea at 45 days of growth improves plant vigour and prevent onslaught of early blight and other leaf spots. Fungicides such as maneb, zineb, mancozeb , captafol, chlothalonil provide good control the disease. First spray should be applied as soon as lower leaves develop the spots which coincide with the secondary spread of the disease.

Franc *et al*. (1988) have developed a prediction model based on accumulated day-degree temperature above 7.2 ºC from planting. This model forecasts beginning of secondary spread of the pathogen. Fungicides used according to the forecast model helps in reducing the use of fungicides. An integrated management of both early and late blight by combined application of fungicides have been suggested by Shtienberg (2001).

Resistance to *Alternaria solani* is available in *Solanum phureja* and *S. chacoense.* It can be exploited in breeding varieties resistant to early blight. A few varieties such as Kufri Sindhuri show good resistance to early blight. Four synthetic peptides, viz. pep 6, pep 7, pep 11 and pep 20 have been found to inhibit both *A. solani* and *P. infestans* on potato leaves. Expression of these peptides in transgenic potato plants could lead to enhanced disease resistance against these pathogens (Gul-Shad-Ali and Reddy, 2000).

2.3. Black Scurf and Stem Canker

Black scurf of potato is an other serious disease of potato worldwide. It affects appearance, size, shape of potato tubers, reduces crop stand, quality and price of the produce. Yield losses up to 35 %, mainly due to sprout injury, resulting in gaps in germination have been reported in some locations (Banville, 1978).

2.3.1. Symptoms

Black scurf on tubers and stem canker are two distinct phases of the disease. The most common symptoms are on tubers as black irregular lumpy encrustations of fungal sclerotia which stick to the surface of tubers (Fig. 6). Other symptoms on tubers in serious cases could be cracking, malformation, pitting and even stem end necrosis. Shortly after planting seed potatoes the fungus may attack young sprouts on tubers through epidermis and produce dark brown lesions thereby killing these underground sprouts much before the crop emergence resulting in gappy germination (Dutt, 1979). Reddish brown lesions may develop on stems and often

Fig. 6:   Black scurf on potato tuber

girdle them. Partial or complete girdling of the stems could result in rosetting of plant tops, purple pigmentation, upward chlorosis or rolling of leaves. Formations of aerial tubers in axis of leaves, due to interference with starch translocation, are also observed in the infected plants. The sexual or perfect stage appears on infected stems just above soil line as whitish grey mat or mycelial felt. These mats are often located above a lesion on the below ground portion of stem.

2.3.2. Pathogen

The imperfect stage of the pathogen is *Rhizoctonia solani* Kuhn and the perfect basidial stage is *Thenatephorus cucumeris* (Frank) Donk (syn. *Corticum vagum* Berk. & Curt. or *Pellicularia filamentosa* (Pat.) Rogers, or *Hypochnus solani* Prill.& Delacr. (Walker, 1969). *R. solani* exhibits much variability in cultural characteristics and hyphal fusions called antastomosis. The fungal isolates have been classified according to anastomosis groups. Twelve such groups have been recognized (Carling *et al.*, 1994). The most common pathogenic isolate inciting black scurf on tubers and canker on stem belongs to group AG–3 (Parameter *et al.*, 1970; Virgen-Calleros *et al.*, 2000). Eight subgroups have been identified within group AG-3 based on variations in isozyme patterns (Laroche *et al.*, 1992; Carling, 1996). The isolates have also been characterized on basis of sclerotial patterns and cultural characteristics (Raj *et al.*, 1974).

Mycelium of the pathogen is generally dark brown in colour. The hyphae are large multinucleate and branch near distal septum of the cell. They show right angle branching and constriction at the point of origin and a prominent septal pore. Early infection are initiated by differentiation of hyphal tips to T–shaped branches followed by formation of cushion like structure and development of appressoria from where thin infection hyphae arise and penetrate the underlying

stem or stolon tissues. These infection sites (cushions) serve as additional food basis for the pathogen and are prerequisite for development of lesions on stems or stolons (Hofman and Jongebloed, 1988; Keijer *et al.*, 1996). Exudates from tubers stimulate development of pathogen. Mycelial encrustation on tubers develops into black scurf sclerotia (Dijst, 1990; Christias and Lockwood, 1973). The pathogen produces a growth regulating toxin that may be partially responsible for tuber malformation.

2.3.3. Epidemiology

Rhizoctonia solani survives as sclerotia on seed tubers or as mycelium in plant debris in soil or on alternate hosts. The pathogen has a wide host range including many Solanaceous and non solanaceous plants. But the main sources of inoculum are infected seed tubers and infested soil. Sclerotia of the pathogen germinate between 8 to 30 °C and invade emerging sprouts or potato stems. Optimum temperature for germination of sclerotia is 23 °C. Optimum temperature for development of stem lesions is 18 °C (Walker, 1969). Sclerotial development on tubers is initiated depending on environmental conditions. Maximum development of sclerotia takes place in the period between dehaulming and harvest of the crop. Late harvested crop show more black scurf incidence.

2.3.4. Management

Pathogen survives both in soil and on tubers and the disease hence can be managed the best by following the suitable (recommended) cultural practices together with seed disinfestations. Soil solarization with transparent polyethylene mulching during hot summer months in Indian subtropical plains was found effective for control of the disease (Arora *et al.*, 1997). A combination of soil solarization and seed treatment with 3 % boric acid or *Trichoderma viride* further improved efficacy of disease controls in *Rhizoctonia* infested soil (Arora, 2000b, Munoz–Ruiz *et al.*, 2001). Shallow covering of seed tubers allows less opportunity for the fungus to attack the susceptible sprouts and consequently less disease incidence. Two to four year crop rotation with cereals and legumes reduces the disease. Seed treatment with 3 per cent boric acid as atomized application on infected tubers was found more economical than the dip treatment for control of seed inoculum (Khanna and Sharma, 1996). Fungicides such as benomyl, thiabendazole, carboxin, pencycuron and azoxystrobin (Virgin-Callerus *et al.*, 2000), fenpiclonil (Welsh and Callaghan, 1996) are effective for control of the disease. Biocontrol agents such as *Trichoderma viride* (Arora, 1999), *T. harzianum* (Mishra *et al.*, 2000), *Bacillus subtilis* (Schmiedeknecht *et al.*, 1998), non pathogenic binucleate *Rhizoctonia* (Tsror *et al.*, 2001), *Trichoderma atroviride* (Huang-Mc Beath, 2001), *Gliocladium virens*, *G. catenulatum* and others have been identified to be effective against *R. solani*. A bioformulation developed from *T. viride* was found very effective for control of the disease when used as seed treatment before planting potatoes (Arora and Somani, 2001). Biocontrol products such as 'Primastop' developed in Finland (Niemi and Lahdenpera, 2000) and 'Stifun' in Russia (Yakhin *et al.*, 1998) have also been reported to be effective.

2.4. Fusarium Dry rots

Dry rot of potatoes caused by *Fusarium* spp. is an important storage disease distributed world wide (Boyd, 1972). The disease affects tubers causing wilted plants in field. Tubers become infected through wounds during harvest, handling and transport but the symptoms become evident only after 2 to 3 months of storage. Planting unsuberized cut pieces of potato tubers result in Fusarial seed piece decay. *Fusarium* spp. present in soil infect the cut surfaces of the seed pieces which rot from surface inward eventually destroying growing buds and result in poor emergence of the crop. Under such certain conditions losses by Fusarial rots may go up to 50 % (Whitehead *et al*. 1953; Hudson and Orr, 1977).

2.4.1 Symptoms

The disease on tubers is generally visible about a month after storage as small brown lesions on surface of affected tubers. The lesions subsequently enlarge, wrinkle and form concentric rings. In later stages of infection, a cavity may become visible in the centre of concentric rings and whitish to pinkish or dark brown growth of fungal mycelium also develops (Fig. 7). Rotten tubers shrivel and get mummified. Under high relative humidity the secondary organisms such as *Erwinia*
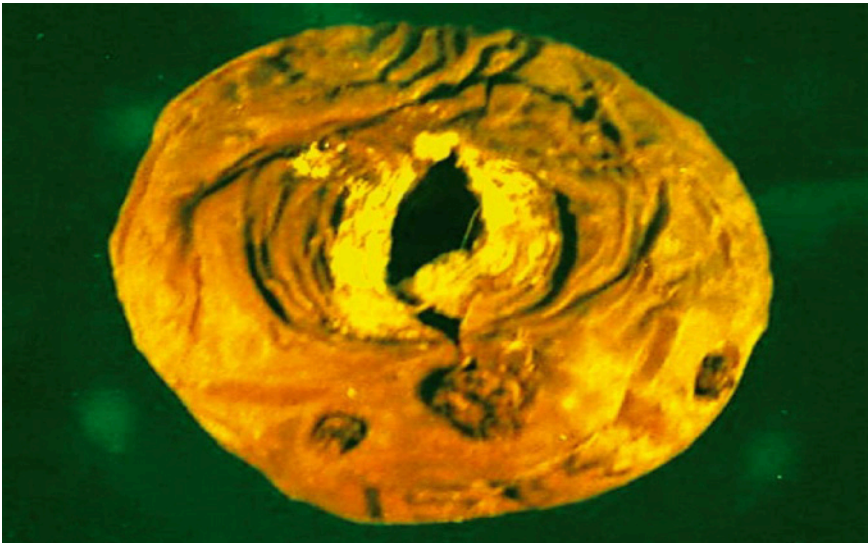
Fig. 7:   Dry rot of potato

spp. invade the infected tubers causing combined infection of soft rot. Exudates containing bacteria come out from such mixed infected tubers resulting in soft rot in surrounding healthy tubers as well.

2.4.2. Pathogen
As many as 14 or more species of *Fusarium* infect potato tubers and cause dry rot. *Fusarium oxysporum* Schlechtend (Fr.), *Fusarium solani* (Mart.) Sacc.; *F. eumartii* (Carpenter) Snyder & Hans; *F.coeuleum* (Lib.) Sacc.; *F.eqiseti* (Corda) Sacc.; *F. redolens* Woolenweb, and others have been reported to be associated with dry rots. However, *F. oxysporum* and *F. solani* are encountered frequently. White fluffy mycelial cushions develop on surface of the infected tubers. Mycelium of the pathogens consist of branched and septate hyphae which are present inter or intra cellular in the host tissue. Conidiophores arise from the mycelium and produce 1 to 4 celled sickle shaped conidia of variable size. Chlamydospores which survive unfavourable conditions may also develop in pustules. These may be intercalary or terminal. The fungus remains viable in soil for 9 to 12 months. Both *F. oxysporum* and *F. solani* have good saprophytic ability to survive in soil (Mall, 1977).

2.4.3. Epidemiology
Pathogenic 'Fusaria' are always present in soil, air, on implements, containers and it is not easy to eradicate them. They can not infect intact periderm and lenticels of tubers but cuts and wounds met during harvest, grading, transport and storage predispose them to infection. An interval of 10 - 15 days between haulm destruction and harvest increases strength of tuber skin and thus reduce dry rots. However, reduction in dry rot by increasing interval between haulm destruction and harvest was not supported by observations of Carnegie *et al*. (2000). Use of herbicide paraquat used after dehaulming of crop has been observed to increase dry rots (Somani *et al*., 1995). Dry rot development is affected by tuber damage, degree of curing, tuber size and storage conditions. Susceptibility to tubers increase with tuber age. The pathogen enters the tubers through wounds and proper wound healing reduces the infection. Tubers cured for wound healing at 21 ºC with adequate aeration develop wound periderm in 3 to 4 days but it takes more time at lower temperature. Development of disease is also affected by moisture and temperature. The fungus grows well between 15 to 28 ºC. *F. oxysporum* has been reported to become non- pathogenic below 10 ºC (Agarwal, 1949). However, disease development continues at low temperature in cold stores. Storage period and relative humidity have been found to be positively correlated with dry rot while the maximum temperature is negatively correlated (Singh, 1986). Large sized tubers are more susceptible than small tubers. No significant correlation exists between chemical composition of tubers and susceptibility to dry rots (Singh, 1986; Percival *et al*. 1999). Some volatile compounds are produced by dry rot affected tubers and an early warning system using sensors to detect these volatile compounds, has been developed (Lacy–Costello *et al*., 2001).

2.4.4. Management

Avoiding bruises by careful handling of the produce would minimize dry rots. This can be done by delaying harvesting for about two weeks after haulm destruction when skin of the tubers have matured. Bruises can also be avoided by taking suitable precaution with machinery, proper adjustment, padding etc. of the equipments. Harvesting on cold frosty morning predisposes the tubers to bruises. Washing tubers to remove contaminated soil that adhere the tubers and drying these in shade reduces the risk of infection (Small, 1945). Harvested potatoes should be stored at around 13 to 18 ᵒC and moderate humidity for two to three weeks for bruises to heal before putting the potato to cold stores. Seed treatment with 1% formaline for half a minute was found effective against dry rot (Boyd, 1947). Spraying tubers with 1200ppm thiabendazole or benomyl checks the disease (Leach, 1975; 1976; Leach and Nelson, 1975). But, resistance to thiabenazole in Fusaria has also been encountered (Hanson *et al.*, 1996). Avoiding planting of cut tubers or treating the cut tubers with dithiocarbamates reduce Fusarial seed piece decay (Rich *et al*. 1960). Control of Fusaria through biocontrol agents such as *Trichoderma* spp. (Pinzon *et al.*, 1999; *Pseudomonas fluorescens* (Schisler *et al.*, 2000; Kim ZinWoo *et al.*, 1998), *P. aeruginosa* (Gupta *et al.*, 1999), *Bacillus subtilis* (Kim-Byung Sup *et al.*, 1995) have been found effective.

Commercial biopreparations from *P. florescens* have been developed (Ermakova and Shternshis, 1994). Combination of biocontrol genera *Enterobacter* and *Pseudomonas* and 2 chitnolytic enzymes from *Trichderma harzianum* had inhibitory effect on spore germination of *F. solani* (Lorito *et al*. 1993) which indicate a possibility that certain bacteria capable of binding to fungal cell walls and expressing fungal genes coding cell wall degrading enzymes may act as powerful biocontrol agents. Transgenic potato plants constitutively over expressing β 1-3—glucanase gene from *Nicotiana plumbagi- nifolia* for resistance against *F. oxysoporum* have been developed (Libantova *et al*. 1998).

2.5. Wart

Wart occurs in Africa, Asia, New Zealand, Europe, North and South America (Lapwood and Hide, 1971; O'Brien and Rich, 1976). It caused great damage to potato in Europe until immune varieties were introduced. The disease once established is difficult to eradicate since the resting spores of the pathogen may remain viable for 20 to 30 years or longer.

2.5.1. Symptoms

Rough warty mostly spherical outgrowths or protuberances appear on buds and eyes of tubers, stolons, or underground stems or at stem base. Wart may appear occasionally on above ground stem, leaf or flowers. Underground galls are white to light pink when young and turn brown or light black with age. Above ground

galls are green to brown or black. The wart tissues are soft and spongy. Tubers may turn completely warty which desiccate or decay at harvest (Fig.8).

Fig. 8: Wart disease on potato plant

2.5.2. Pathogen

Wart is caused by *Synchytriun endobioticum* (Schilberszky) Percival a member of Chytridales. It is an obligate parasite with at least 10 pathogenic races (Lapwood and Hide, 1971). The fungus lacks mycelium and has a thin walled summer sporangium stage and a thick walled 'winter' or resting sporangium stage. Both summer and winter sporangia produce an extended vesicle called sorus from where zoospores are produced. The zoospores are pear shaped and possess a posterior flagellum. Potato is the main host although experimentally a number of Solanaceous species gets infected upon artificial inoculation (Phadtare and Sharma, 1971).

2.5.3. Disease Cycle and Epidemiology

Warty growths disintegrate releasing abundant resting sporangia in soil. These sporangia can remain viable in soil for more than 2 decades even in the absence of suitable hosts. The resting sporangia under wet soil conditions and temperature between 10 to 27 ºC germinate to release haploid uni-nucleate zoospores. The zoospores swim in soil, encyst and infect epidermal cells of meristematic tissues of growing buds, stolons tips or leaf primodia by means of an infection peg within 1 to 2 h of their formation. After successful infection a uni-nucleate thallus develops within the infected cell which enlarges to form a pro-sorus. A vesicle develops from prosorus and contents of the prosorus pass on to the vesicle to form a sorus

within an infected cell. The sorus divides repeatedly to form several sporangia in which zoospores develop. Finally wall of the sorus breaks releasing sporangia and zoospores in soil. New infection results from the zoospores. This process continues throughout the growing season. Growth of the pathogen fungus within the host stimulates hypertrophy and hyperplasia of neighboring host cells without actively infecting them which result in an increase in meristematic activity and development of warts of variable size depending upon the degree of stimulation (Lapwood and Hide, 1971).

Under certain conditions possibly under water stress (Curtis, 1921), the haploid zoospores fuse in pairs to form a zygote. The zygote invades the host tissue and develop into thick walled 'winter sporangia' which are echinulated with prominent exterior ridges. These winter sporangia or resting spores are released in soil with decay of warty growths and serve as primary inoculum. Wart is favoured by periodic flooding followed by drainage and aeration since free water is required for germination of sporangia and dispersal of zoospores. Optimum temperature for germination of resting sporangia to zoospores is between 14 to 24 ºC. Both summer sporangia and resting spores can germinate between 12 to 28 ºC. The pathogen spreads from one locality to another through infected seed tubers, soil adhering tubers, contaminated manure, machinery and other carriers. Mean temperature below 18 ºC and annual precipitation of about 70cm favour disease development.

2.5.4. Management

The disease has been successfully managed by sanitation, long crop rotation, growing resistant and immune varieties and by enforcing strict quarantine legislation in countries of EPPO region (McNamera and Smith, 1998), Canada (Hampson, 1993), Maryland USA (Putnam and Sindermann, 1994) and India (Singh and Shekhawat, 2000). Periodic surveys are required to monitor viability of the pathogen in soil and efficiency of the quarantine measures.

An effective control of the disease is easily possible by mere cultivation of wart immune varieties. Many varieties wart immune have been developed throughout the world. In resistant varieties the pathogen infects the plants but development of symptoms is suppressed while in the immune varieties a hypersensitive reaction occurs upon infection with zoospores which get killed in the process. Development and introduction of wart immune varieties such as Kufri Jyoti, Kufri Bahar and Kufri Sherpa (Sharma *et al*. 1976), Kufri Kanchan (Phadtare *et al*., 2000) to wart infested region of Darjeeling Hills coupled with domestic quarantine had great impact in containing the wart in the region (Singh, 1998).

Application of fungicides and chemical to soil is costly and not a practical approach (Hodgson *et al*. 1974; O'Brien and Rich, 1976). Amendment of infested soils with 4 and 8 % crabshell (w/w) however, in small plots reduces population of the pathogen (Hampson and Coombs, 1995). Intercropping potato with maize, or growing rotational crops such as bean and radish has been found to reduce pathogen population (viable resting spores) in soil (Singh & Shekhawat, 2000).

2.6. Powdery Scab

Powdery scab is an important disease of potato in temperate climate and high altitude in the tropics (Hines, 1976). The disease has been reported from Africa, Asia, Australia, Europe, North and South America (Walker, 1969). The disease causes scab like lesions on tubers and markedly reduces its market value if the incidence/ index is high.

2.6.1. Symptoms

The disease is confined to below ground parts of potato plants. Symptoms on potato tubers first appear as purplish brown sunken lesions which later turn to scab like lesions but unlike common scab the lesions of powdery scab are round, raised, filled with powdery mass of spores and surrounded by ruptured remains of epidermis (Fig. 9). The powdery mass consists of cytosori or spore balls. Each spore ball contains many spores which adhere to one another along with their walls. Under certain conditions wart like protuberances may develop (O'Brien and Rich, 1976; Bhatacharryya and Raj, 1978. The infected tubers may shrivel or develop dry rot like symptoms in storage. Powdery scab pustules also predispose the tubers to *P. infestans* pathogen (Bonde, 1955). The pathogen may also serve as soil borne vector of potato mop top virus (O'Brien and Rich, 1976).

2.6.2. Pathogen

Powdery scab is caused by *Spongospora subterranea* (Wallr.) Lagerheim. It is a member of order Plasmodiophorales. The spores of the pathogen are yellow to

Fig. 9:   Powdery scab on potato tuber

brown, thin walled, polyhedral, uni-nucleate structures which germinate to produce a single primary zoospore.

2.6.3. Etiology and Epidemiology

The pathogen survives winter as sporangia in infected potato tubers. It can also survive in soil up to six years. The pathogen can survive passage through animal digestive tract and manure from animals that had ingested infected tubers, and can serve as a potential source of inoculum. The zoospores of the pathogen penetrate roots, stolons, tubers and produce a multinucleate sporangial plasmodium in the host. In roots the plasmodium produce sporangia which further produce up to 8 secondary zoospores. The zoospores reinfect the host tissue and several such generations of zoospores may be produced in a single season under ideal environment. In tubers, the plasmodium produces resting spore which can over winter and persist in tuber and soil for a long period.

2.6.4. Management

Planting of disease free seed obtained from disease free area helps in management of the disease. Seed treatments are not effective. The disease can be minimized in field by avoiding flooding through proper drainage. Crop rotation with non solanaceous hosts is effective. Resistant cultivars have been developed in Germany, Russia and Chile (Manzer *et al.*, 1964).

2.7. Charcoal Rot

Charcoal rot is also an important disease of many vegetable crops in tropical and subtropical countries where high soil moisture is coupled with temperature exceeding 28 °C (Chupp and Sherf, 1960). It is of major importance in the Mediterranean region, Hawaii, Southern United States of America, warmer areas of Peru and India (O' Brien and Rich, 1976). The disease can cause severe losses under unusually warm wet weather. The affected tubers rot in field and during storage.

2.7.1. Symptoms

Early symptoms on tubers develop around eyes, lenticels and stolon end where a dark light grey, soft, water soaked lesion develops on the surface. Cavity within the lesion becomes filled with black mycelium and sclerotia of the pathogen. Secondary organisms may develop in such lesions especially under wet conditions causing significant losses (Pushkarnath, 1976). Under low moisture the lesions may shrink and dry rot type symptom may develop at harvest and storage. The fungus also attacks stems exhibiting stem blight or shallow rot similar to black leg which cause the foliage to wilt and turn yellow.

2.7.2. Pathogen

Charcoal rot of potato is caused by fungus *Macrophomina phaseolina* (Tassi) Goidanich Syn. *M. phaeoli* Maubl.(O'Brien and Rich, 1976). Black, smooth, hard 0.1 to 1.0 mm sized sclerotia of the fungus develop within roots, stems, tubers and leaves. The perfect stage of the fungus is considered to be *Botryodiplodia solanituberosi* Thiram. (Thirmalachar and O'Brien, 1977) which may develop in stems of potato, jute, sun hemp and maize. Pycnidia may develop on leaves and stems depending upon the strain of the fungus. Conidia are single, hyaline and ellipsoid to obovoid.

2.7.3. Epidemiology

M. phaseolina is a weekly parasitic soil fungus and over winters in soil as sclerotia in plant debris, weeds and alternate host crops. Both soil and infected tubers serve as source of inoculum. Temperature around 30 °C is optimum for growth and infection of the fungus. Poor plant nutrition and wounds predispose the plants to charcoal rot. Temperature around 30 °C or above are very favourable for the disease development, the rot is slow at 20 to 25 °C and stops at 10 °C or below. Fungal growth stops in tubers placed in cold stores but resumes soon after taking the tubers out of cold stores.

2.7.4. Management.

Soil temperature preceding harvest is crucial for disease development. Planting early maturing cultivars and harvesting before soil temperature exceeds 28 °C (which is around middle of February in eastern plains of India) provide good control of charcoal rot (Thirumalachar, 1955). Frequent irrigations after middle of March in eastern Plains of India keep down the soil temperature and reduce the disease incidence. Rotation with non host crops and use of seed from disease free area, avoiding cuts and bruises at harvest reduce disease incidence.

Resistance against charcoal rot has been located in certain clones of *Solanum chacoense* and may be utilized in breeding resistant varieties. Bio-control using *Bacillus subtilis* through seed treatment has been reported to reduce incidence of charcoal rot (Thirumalachar and O'Brien, 1977).

3. Bacterial Diseases

Bacteria form an important group of pathogens of potato. They attack the plant primarily through wounds but may also enter through a natural opening such as stomata and lenticels. Warm moist conditions favour bacterial growth. Most important bacterial diseases, having major threat to potato production, are bacterial wilt, soft rots and common scab.

3.1 Bacterial wilt

Bacterial wilt or brown rot is a destructive disease of potato especially in tropical and subtropical parts of Asia, Africa, South and Central America (O'Brien and Rich, 1976; He, 1986, Machmud, 1986; Shekhawat *et al*., 2000). Losses up to 75 % have been recorded under extreme conditions (Gadewar *et al*., 1991).

3.1.1. Symptoms
Initial symptoms of the disease are slight wilting or drooping of foliage especially at end of branches during hot period of the day which recover at sunset. Later the leaves turn yellow and the wilting becomes permanent leading to collapse of the plant (Fig.10). Cross section of the stem reveals browning of vascular bundles and bacterial slime oozes out of the vascular region. The disease also affects potato tubers. The first symptoms on tubers include brownish vascular discoloration extending to eyes and other buds. Bacterial mass may also emerge through the affected eyes (Fig. 11) to which soil adheres at harvest. Freshly cut tubers when squeezed show glistening droplets of bacterial ooze emerging from the vascular ring.

3.1.2. Pathogen
Bacterial wilt or brown rot is caused by *Ralstonia solnacearum* (Smith) Yabunchi *et al*. (Yabunchi *et al*., 1995). Earlier the pathogen was called *Pseudomonas solanacearum* (Smith). The bacteria is gram negative, rod shaped, measuring 0.5

Fig. 10: A bacterial wilt affected potato plant in field

Fig. 11: Bacterial ooze from the eye of a brown rot infected potato tuber

x 2.5 um, non spore forming, non encapsulated, nitrate reducing, ammonia forming and aerobic. It is sensitive to desiccation and has low tolerance to sodium chloride (up to 2 %t) as compared to other species of *Ralstonia*. The pathogen under oxygen stress conditions in culture media shift to avirulent form. Lipopolysacchrides of the pathogen play an important role in determination of virulence (Hendrick and Sequira, 1984). Virulent isolates are mainly non flagellate and thus non motile whereas avirulent forms bear 1 to 4 polar flagella and are motile (Kelmen and Hruschka, 1973). Virulent isolates on tetrazolium chloride medium develop fluidial irregular shaped colonies with white to pinkish centre (Kelman, 1954) where as avirulent types produce small round, dark red dry colonies. Optimum growth of the pathogen occurs between 28 to 32 °C. *R.solnacearum* is classified into five races based on host range and five biovars based on carbohydrate metabolism (Hayward, 1964; Buddenhagen and Kelmen, 1964). Potato is affected mainly by race 1 biovar I, III, and IV and race 3 biovar II. Differences in geographical distribution of the biovars have been observed (French, 1979). The pathogen produces extracellular polysaccharide (Husain and Kelmen, 1958) and a phytotoxic glycopeptide toxin (Gowda and Rai, 1980) causing wilt of infected plants. The pathogen has a wide host range and affects more than 200 plants species including both cultivable and weed hosts. Rapid methods to detect pathogen in potato have been developed (Priou *et al*., 1999; Elphinstone *et al*., 2000; Lyons *et al*., 2001; Weller *et al*., 2001).

3.1.3. Epidemiology
Infected tubers and plant debris in infested soil are two major sources of inoculum. The pathogen infects roots of healthy plants through wounds. Nematodes such as

Meloidogyne incognita which affect potato roots and tubers increase wilt incidence (Nirula and Paharia, 1970). Inoculum potential of about 10^7c.f.u. /g soil favours infection (Devi *et al.*, 1982) which however is dependent on other predisposing factors. Mean soil temperature below 15 and above 35 °C do not favour the disease development (Keshwal, 1980). High soil moisture, temperature, oxygen stress and soil type affect the survival of the pathogen (Shekhawat *et al.*, 1978; van Elsas *et al.*, 2001). The pathogen population decline gradually in soil devoid of host plants and their debris. Transmission of *R.solanacearum* from one area to another mainly occurs through infected seed or irrigation water, and farm implements (Khanna and Vishwadhar, 1974; Shekhawat *et al.*, 1988a; Pradhanang, 1999; Elsas *et al.*, 2001).

3.1.4. Management

An integrated approach involving use of pathogen free seed potato, reduction of field inoculum, growing crop under right environmental conditions, chemical and biological control has helped in control of the disease (Persson, 1998; Schans and Stooghs, 1998; Lemaga, 2001).

Incidence of bacterial wilt declines by application of bleaching powder @ 12 kg/h mixed with fertilizer or soil drenching after first earthing up (Shekhawat *et al.*, 1988a,b) and use of healthy seed (Gadewar *et al.*, 1991; Hayward, 1991). Soil amendments with urea or borax or boric acid (Lee *et al.*, 1982) or application of copra and pea nut meals controls the disease (Shekhawat *et al.*, 1982b). Biocontrol of bacterial wilt by use of antagonists such as *Pseudomonas flourescens*, *Bacillus* spp, avirulent *P. solanacearum* and actinomycetes have been found to be effective (Shekhawat *et al.*, 1993a; Mclaughlin *et al.*, 1988).

Breeding for resistance have not been very successful especially under subtropical and tropical highlands. Cultivars derived from *S. phureja* exhibiting resistance under cool highland subtropics, succumbs to the disease under high temperature prevailing in the tropics.

3.2. Bacterial Soft Rot and Black Leg

Bacterial soft rot of potato is found wherever potatoes are grown. The disease affect the crop at all stages of growth. It causes soft rot of tubers at harvest, transit, storage and blackleg of foliage during the crop growing season. Losses under poorly ventilated storage or transit may go up to 80 % (Somani and Shekhawat, 1990; Cromarty and Easton, 1973).

3.2.1. Pathogen

A number of pectolytic bacteria, *viz. Erwinia carotovora* ssp. *carotovora* (Jones) Bergey, Harrison, Breed, Hammer & Huntoon; *E. carotovora* ssp. *atroseptica* (van Hall) Dye; *E. chrysanthemi* Burkholder *et al*; *Bacillus polymyxa*; *B. subtilis*;

B. mesentericus; *B. megaterium* de Bary; *Pseudomonas marginalis* (Brown) Stevens; *P. viridiflava* (Burkholder) Dowson; *Clostridium* spp; *Micrococcus* spp.; and *Flavobacterium* have been found to cause soft rot. *Erwinia* and *Clostridium* are active under temperate climate while *Bacillus* and *Pseudomonas* are actively involved under tropics and subtropics.

Erwinia are gram negative bacteria, rod shaped with peritrichus flagella. They can grow both under aerobic and anaerobic conditions, produce pectolytic enzymes and degrade pectin in middle lamella of host cells, breakdown tissues causing soft rot and the decay. The decaying tissue become slimy and foul smelling and brown liquid oozes out from the soft rot affected tubers. About 1500 strains of pectiolytic *Erwinia* have been isolated from infected plants and tubers (Sledz *et al*., 2000).The pathogen produces certain volatile compounds such as ammonia, trimethylamine and several volatile sulfides (Lacy *et al*., 1999) and early detection of such volatile compounds in storage could be used as a method to detect the disease at initial stage (Lyew *et al*., 2001).

3.2.2. Symptoms

On tubers, the disease may appear as small soft water soaked spots around lenticels which enlarge under high humidity or shrivel and get sunken under dry conditions (Fig 12). The pith of infected tuber decay beyond the boundary of external lesion, turn cream to tan brown in colour and the tissues becomes soft and granular. A brown to black pigment may develop around the lesion. Immature, large tubers bruised at harvest, tubers infected with late blight and those grown with high nitrogen fertilizer especially ammonium chloride have been found to be more prone to soft rot (Bennett, 1946; Smith and Ramsey, 1947; Walker, 1969; Perombelon and Kelman, 1980; Somani and Shekhawat, 1988).

Fig.12: Soft rot of potato tuber

'Black leg' symptoms appear on foliage at any stage of plant growth but are more frequent in dense canopies under warm and wet weather. The disease develops from soft rot affected tubers. A soft black lesion appears at base of stem which extend to decaying soft rot affected seed tuber in soil and up to a little above ground level. Tissues in the lesion shrivel and rot. The affected plants become stunted, exhibit yellow chlorotic foliage, wilt and die without producing fresh tubers. Occasionally, necrosis of leaf vein, brown to black lesions on petioles and succulent stems may appear. On stems and petioles, the lesions enlarge into stripes, envelop the affected tissue and cause soft rot and toppling of stems and leaves (Perombelon and Kelman, 1980; Somani and Shekhawat, 1990).

3.2.3. Epidemiology

Bacteria that cause soft rot and black leg are carried in lenticels, tuber wounds and even on their surface and spread to healthy tubers in stores, while cutting, handling or planting seed tubers (Perombelon and Kelman, 1980, Weber, 1990). Insects, especially maggots of *Hylemyia* spp., may also transmit the bacteria from one tuber to another (Agrios, 1969). The bacteria may also be carried latently in tubers without any visible symptoms (Piplani *et al*., 1983). Water film on tuber surface leads to proliferation of lenticels and also creates anaerobic conditions as well as other injuries on tuber surface predispose potatoes to soft rot (Tripathi, 1979).

From soft rot infected seed tubers, bacteria may enter vascular tissues of developing stems to incite black leg disease under favourable conditions. From black leg infected plants, the pathogen can reach daughter tubers through stolons and initiate tuber decay at the site of tuber attachment (Shekhawat *et al*, 1984). Decaying tubers in soil could serve as source of contamination for healthy tubers. The pathogen may also spread while washing of the produce with contaminated water (Dartz, 1999). The threshold level for disease development is about 103 cells of *E.carotovora* ssp. *atroseptica* per tuber (Perombelon, 2000).Tubers harvested in wet soil, poor ventilation in transit and storage promotes the rot (Hingorni and Andy, 1953).

3.2.4. Management

Planting whole seed tubers or well suberized seed pieces in a well drained soil, with temperatures around 10 to 13 °C, at less planting depth help reduce incidence of black leg. Application of stable bleaching powder before planting is helpful as it increases emergence and yield (Parashar and Sindhan, 1988; Karwasra and Parashar, 1998). Application of bleaching powder with the last irrigation to the crop also reduces soft rot of storage in storage (Parashar *et al*., 1986). Sanitation or applying a disinfectant to equipments while cutting tubers helps reduce soft rot. Planting of susceptible cultivars in wet soil, or an irrigation before emergence of the crop from seed pieces should be avoided, as these factors increase risk of seed piece decay. Calibration of equipment to minimize bruises during harvest, avoiding exposure of tubers to sunlight, proper aeration in transport and storage

are also some of the measures that help in reducing soft rot incidence. Tuber treatment with 3 % boric acid (Somani and Shekhawat, 1985) or 0.05 % copper sulphate (Zhang *et al*., 1993) or 160ppm Kasugemycin also reduce the incidence.

Crop rotations like green manure-potato– wheat reduce soft rot (Shekhawat *et al*., 1984). Varieties / genotypes resistant to soft rot have been identified in several countries (Tripathi and Verma, 1975; Reeves *et al*., 1999; Zimnoch *et al*. 1999).

Control of soft rot through some essential oils and extracts of hemp flower and common weeds (Kerbs and Jaggi, 1999; Vijaypal *et al*., 1993) or microbial antagonists such as strains of *Pseudomonas putida* and *P. flourscence* (Abdelghafar and Abdelsayed, 1997; Kastelein *et al*., 1999), *Bacillus subtilis* strain BS107 (Sharga and Lyon, 1998) and *Erwinia herbicola* Eh 252 (Vanneste *et al*., 1994) has been reported to be effective.

Transgenic potato plants, modified with chimeric genes encoding PL 3 of *E.carotovora* ssp. *atroseptica* under control of patatin B 33 gene promoter and cauliflower mosaic virus (CAMV) 35 S, were investigated for resistance against soft rot and transgenic lines which synthesized PL 3 were found more resistant to tissue maceration by *E. carotovora* and its enzymes (Wegener *et al*., 1996).

3.3. Common Scab

Common scab occurs in most potato growing areas in Africa, Asia, Europe, North and South America. The disease cause superficial lesions on surface of potato tubers and affects quality of the produce. The affected tubers fetch low price in market due to their bad look and also because deeper peeling is required before consumption. In India, seed lots exceeding 5 % incidence is rejected by seed certification agencies causing huge loss to seed industry (Shekhawat *et al*., 1999; Shrivastava and Sahai, 1997).

3.3.1. Symptoms

Scab lesions on tubers may be shallow, raised or sunken. Symptoms on young tubers begin as inconspicuous round minute brown specks, less than 1mm in diameter under stomata or young lenticels. The lesions are initially shallow and typically circular with definite margin (Taylor and Decker, 1947; Paharia and Pushkarnath, 1963). As the lesions increase in size, the periderm cracks due to formation of cork layer around lesions which assume various shapes such as reticulate and may be shallow or deep pitted. The lesions may coalesce to affect large area on tuber surface. The lesions on mature tubers may be mere abrasions; star shaped with corky depositions; concentric wrinkled layers of cork around a central black core; raised and rough corky pustules or 3 to 4 mm deep pits surrounded by hard corky tissue (Fig. 13), (Nagaich and Dutt, 1972; Jeswani *et*

Fig.13: Common scab of potato showing deep pitted star like lesions

al., 1987). Tubers which protrude above ground are not invaded (Gram and Weber, 1953) and tubers which grow faster than other develop higher infection (Jones, 1922). Quick bulking varieties in general suffer more than slow bulking varieties (Vashist and Chaubey, 1979; Mohanty *et al.*, 1980; Shekhawat *et al.*, 1993).The tuber lesions being corky and hard are generally not affected by secondary organisms and do not affect storage life of the produce.

3.3.2. Pathogen

Many *Streptomyces* spp. may cause common scab (Liu *et al.*, 1996). The prominent among them are *Streptomyces scabies* (Thaxter) Lambert and Loria, *S.acidiscabies* Bambert and Loria, *S. turgidscabis* Takeunchi, *S. collinus* Lindenbein (Dey et al., 1981); *S. griseus* (Krainsky) Waksman & Henria (Dey and Singh, 1983; Jeswani *et al*, 1987), *S. longisporoflavus*, *S. cinereus* , *S. violanceoruber*, *S.alborgriseolus*, *S. griseoflavus*, *S. catenulae* and others. Plant pathogenesis by *Streptomyces* has been reviewed by Loria *et al.* (1997). *Streptomces* are bacteria which resemble fungi due to formation of vegetative substrate mycelium that develop aerial filaments. However, the filaments are of smaller dimensions than the true fungi. These filaments produce spores through fragmentation. *Streptomyces* spp. may be pathogenic or non pathogenic. The pathogenic species produce thaxtomins which are phytotoxins and cause hypertrophy and cell death (Loria *et al.*,1995). Considerable variation exist within the pathogen with respect to their pigment production in media, colour and shape of sporulating filaments and use of specific sugars (Afanasiev, 1937; Leach *et al.*, 1939; Schall, 1940). *S. scabies* form grey, spiral spore chains on several media and produce brown pigment where as *S.*

acidiscabis produce peach coloured wavy chains of spores and brown pigment in medium. Different species of *Streptomyces* have been found associated with various types of scab lesions (Faucher *et al*., 1992; 1993). Boucheck *et al*. (2000) have identified three groups of pathogenic *Streptomyces* which differ in their ecological requirements and produce various symptoms on host under different soil temperature regimes. Better diagnostic assays based on PCR have also been developed to detect the pathogen (Cullen *et al*., 2000).

3.3.3. Epidemiology

The pathogen is both seed and soil borne. It can survive in soil for several years in plant debris and infested soil (Lutman, 1945; Singh and Singh, 1992). Soil conditions greatly influence the pathogen. Favourable conditions include pH between 5.2 to 8.0 or more (Butler and Jones, 1961), temperature in the range of 20 to 30 °C (Gaumann and Hafliger, 1945) and low soil moisture (Sanford, 1926; Singh and Singh, 1981). The pathogen is aerobic in nature and maintaining high soil moisture for 10 to 20 days after tuber initiation helps in reducing the common scab (Lapwood and Hering, 1968).

3.3.4. Management

Seed disinfestation with chemicals is most commonly employed to control the disease. Effective chemicals are methoxy ethyl mercuric chloride, boric acid (Shekhawat *et al*., 1993b; De and Sengupta, 1992).

The disease can be reduced by use of acidic fertilizers such as ammonium sulfate (Huber, 1980), single super phosphate (Grewal *et al*., 1988); and potassium chloride (Heald, 1933). Lower disease incidence has been observed with a higher concentration of water soluble aluminum in soil (Mizuno *et al*. 1998). Application of magnesium and manganese sulfate, and sulfur to potato crop has also been found to reduce the scab (Huber, 1980; Trehan and Grewal, 1980; Vashisth *et al*. 1990; Lambert and Manzer, 1991).

Soil solarization with transparent polyethylene mulching during hot summer season was effective for control of russet scab. The disease severity by this treatment is reduced to almost one third as compared to the unsolarized plots (Arora *et al*., 2002).

Mulching during earlier part of season and frequent irrigation to maintain soil moisture approaching field capacity, from tuber initiating stage to maturity of the crop, has been practiced for control of common scab (Kagawa and Hosaka, 1991; Lapwood et al. 1973; Borowezak and Gladysiak, 1999).

Biocontrol of common scab through antagonists such as *Bacillus subtilis* (Schmiedeknecht *et al*., 1995; 1998), non pathogenic *Streptomyces* spp. (Liu *et al*., 1995; 1996; Lorang *et al*., 1995) and bio-pesticides such as *Geranium pretense* (Ushuki *et al*., 1998) have shown promise.

Rotational crops such as maize, cotton, grain sorghum, wheat, cabbage and onion have also been observed to reduce incidence of the disease (Huber and Watson, 1974; Shekhawat *et al*., 1991).

4.   References

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7

Potato Diseases and their Management

Surinder Kaur and K. G. Mukerji

ABSTRACT: Potato (*Solanum tuberosum* L.) a native food crop, now grown in many parts of the world and next only to cereals in importance, was moved out of its native home in the Andes (South America) only during 15th century. All potato varieties are clones propagated vegetatively by 'seed' tubers and because of this are vulnerable to a wide range of pathogenic organisms, which they transmit from crop to crop. The pathogens may be fungi, bacteria or viruses. Losses can occur when crops are growing, at lifting and when tubers are stored. Some diseases do not destroy tubers, but the surface blemishes they cause decrease marketable value. There is therefore, much emphasis on the production and use of high quality, disease free seed. Though the list of pathogens infecting potato has remained almost unchanged over many decades, there has been steady expansion in the area under potato and improvement in tuber yield/productivity. Obviously, it has been made possible mainly through biotechnology, which make the crop more suitable for genetic manipulations through modern techniques. The potential application of genetic engineering in potato is only limited by human imagination and success stories of expressing new genes bestowing noval traits to potato are regular features. These traits include resistance to viruses, insects, herbicides, abiotic stresses, improvement in quality characters and pharmaceutical possibilities. The main aim of all these developments is to make potato cultivation more efficient, economical and environmentally safe.

1. Introduction

The potato (*Solanum tuberosum* L.) is the most important non-cereal world food crop and is next only to rice, wheat and corn as a major crop in terms of total food production. Among the plant food sources potato contains a better balance of essential amino acid, particularly lysine. Another important point about potato is that it has the capacity to produce more energy and protein per unit land than any other single food crop.

Potato is grown in temperate, subtropical and tropical regions. The potato tuber is composed mainly of water (75-80%). The solids of the tuber are composed of carbohydrates (6-20%) which supply

the energy, fat being negligible (0.1-0.2%) as well as free amino acids. The mineral content of potato is high (0.8-2%), but is low in sodium and high in potassium. The potato contains low fibre (0.6%), and some important vitamins such as C, B and B2 which make it nutritious (Watt and Merill, 1963).

Potato is useful in many ways, 30-72% being used for food (FAO, 1984). It is primarily consumed as a source of carbohydrates in place of rice or wheat, as a vegetable, or in the form of French fries, wafers, chips, mashed and boiled potato, and in soups. It contains a satisfactory mixture of essential amino acids for infant feeding. It is also fed to pigs, and thus returns in terms of fats are enormous. Alcohol prepared from potato supplies a considerable portion of dietary energy. Potato is also being employed to produce fuel alcohol (Bajaj, 1987).

Potato is an annual plant, about 30-100 cm tall and is vegetatively propagated through tubers. The tubers bear the buds, commonly known as "eyes" which sprout on germination and grow into plants. The tubers, the size of which differs with age and cultivar, are grown in fields in ridges to maintain developing tubers under soil because on exposure to light, they become green and unpalatable. The tubers start developing when the plant flowers, and their formation ceases when fruit formation begins. Potato breeding is a difficult task mainly due to tetrasomic inheritance of characters, the high heterozygosity, self incompatibility and male sterility in many cultivars. The conventional methods of potato breeding involve selection, crossing for recombinations and mutations (Hooker, 1983; Ross, 1986). Selection is limited to existing variation, which not only takes a long time but the efficiency of selection is also limited. Thus, according to Wenzel (1980), starting with 100,000 seedlings, it would take 6-8 years to select a desired variety.

The potato is susceptible to many diseases. The fungi that might attack it range from the slime molds to the smuts and rusts. It is susceptible to several viruses of the yellow and mosaic groups, some nonparasitic diseases as black heart, sunscald freezing injury, and a malnutrition caused by deficiency in magnesium, potash and boron may cause damage. Several nematode diseases have been found on it. Diseases of potatoes include arguably the most historically significant crop disease, late blight, which is still the most important potato disease. An increasing emphasis on the cosmetic appearance

of potatoes has recently brought hitherto non significant diseases into prominence. Unless effective methods of control are practiced, serious diseases, such as late blight, ring rot, and leaf roll, can cause the total loss of a crop.

Fungal diseases of economic importance to potato can be broadly categorised into two groups, *viz*. foliar diseases and soil and tuber borne diseases (Large, 1940). Table 1 shows the list of important potato diseases caused by fungi. The most important foliar diseases include late and early blights and Phoma blight, whereas dry rot, common scab, blackscurf, Verticillum wilt and Fusarium wilt are important amongst the soil and tuber borne diseases.

TABLE 1

Some Important Fungal Diseases of Potato

Diseases	Pathogens
Late Blight of Potato	*Phytophthora infestans*
Early Blight of Potato	*Alternaria solani*
Phoma Blight	*Phoma* spp.
Powdery Scab	*Spongospora subterranea*
Wart of Potato	*Synchytrium endobioticum*
Watery Wound Rot	*Pythium ultimum*
Gangrene	*Phoma exigua* var. *foveta*
Silver scurf	*Helminthosporium solani*
Pink Rot	*Phytophthora erythroseptica*
Dry Rot	*Fusarium* spp.
Black scurf	*Rhizoctonia solani*
Skin spot	*Polyscytalum pustulans*
Wilt of Potato	*Verticillium* sp.
Charcoal Rot	*Macrophomina phaseolina*

2. Fungal Diseases

2.1. Late Blight of Potato

Late blight is the most destructive of all diseases of potato. It is caused by the fungus *Phytophthora infestans* (Mont.) de Bary. It attacks and

kills the top of the plant and invades the tubers causing either dry or wet rot. The fungus has the tremendous capacity to adapt to the environment, thus becoming widespread in all environments which support potato cultivation. Despite its name, the first infection often occurs soon after the plants emerge when favourable moisture and temperature prevail. At 70° to 75°F, the fungus grows so fast inside the leaves that within a week after infection, it causes dead spots one half to one inch in diameter. The entire plant may be killed within two weeks.

The blight was first observed in Europe in 1845 at Courtrcu in Belgium in late June to early July. Subsequently, it spread to most of the European countries including U.K. and Ireland, causing the worst ever famine known as 'Irish Potato Famine.' Since then it has spread far and wide. In India it reached through Europe in 1870 and 1880 and was recorded for the first time in Nilgiris Hill (Butler, 1918). The physiology of the fungus did not permit it to reach the plains of India, where the temperature was relatively high for development of the disease. During 1899-1900, it was observed for the first time in the plains in Hooghly district of Bengal. Several outbreaks of late blight were reported in 1912-1913 from Jorhat (Assam) and in 1913 from Rangpur (Bengal) and from Bihar in 1933. Since 1943, the disease has been making regular appearances almost throughout the plains of northern India. However, its epiphytotics are restricted to only certain areas. Intensity of the disease also varies from variety to variety. For example, susceptible varieties like Kufri Chandrmukhi and Kufri Bahar are most prone to blight. Their crop is killed within a period of 8-10 days under blight favourable period, whereas in case of resistant varieties, it takes little longer time.

2.1.1. Symptoms:

The disease affects leaves, stems and tubers. Water-soaked spots or lesions first appear on the leaves during cool, wet weather. The spot appears light green at first and then turn brown. Lesions may also have a yellowish-green margin or halo. A white fungus ring of sporangiophores and sporangia develops on the under surface of the leaves near the margin of the lesions, if the weather remains wet or humid. Blight can progress in infected tubers in potato stores but it does not usually spread to healthy tubers. Blighted tubers are frequently colonised by secondary bacterial pathogens, particularly in poorly ventilated warm humid potato stores. Tubers are then quickly reduced to semi liquid state.

2.1.2. Disease Cycle

P. infestans overwinters as mycelium in contaminated potato tubers. These may be in cull (discarded) piles of potatoes, groundkeepers left from a previous crop of seed stocks. Cull piles are probably the most important source of initial inoculum in the U.K. Diseased tubers give rise to diseased haulm tissue. During suitable weather conditions, sporangiophores are produced which bear many lemon-shaped sporangia. These can be dispersed relatively short distances by rain splash or longer distances on air currents, sporangia require over 90% relative humidity for germination. There are generally two methods of spore germination found in the species, which enable the fungus to adapt itself to a rather wide range of temperature. The higher temperature favours mycelial development, rapid invasion and killing of the plant. However, particularly during slightly cooler, wet weather, each sporangium releases 8-12 motile biflagellate zoospores which encyst and then penetrate tissue directly or occasionally *via* stomata. These zoospores are important in the cycle of contamination of tubers. During wet weather, tubers near the soil surface may be infected by zoospores, which swim in soil moisture. Again zoospores encyst prior to penetrating the tuber *via* wounds of lenticels. Tubers may also become infected if they are exposed to airborne sporangia from haulm tissue or to sporangia and zoospore in the top layer of soil during harvesting. A further interesting feature of the life cycle of *P. infestans* concerns the possibility of sexual reproduction resulting in oospores (Fig. 1). Until recently, only one of the two mating types needed for sexual reproduction could be found in the U.K. However, the complementary mating type, previously confined to Mexico, has been identified in blight infected crops in the U.K. (Tantine *et al*., 1986).
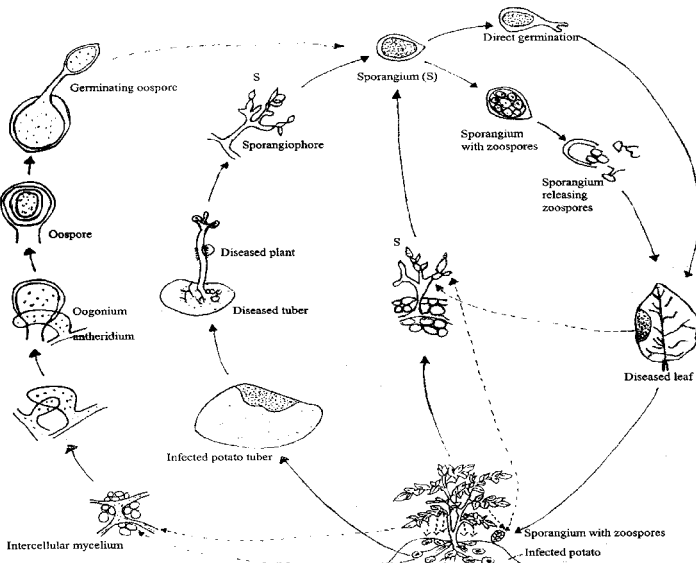
Fig.1.: Life cycle of *Phytophthora infestans*

The fungus infects the potato tubers as well as the tops. Spores from infected tops are carried by rain to the tubers in the soil. Tubers also are readily infected, if they are harvested before the blighted tops are killed, infected tubers quickly show a brown discoloration, which changes to a purplish colour. The fungus usually invades the tuber to about one-fourth to one-half inch below the skin. At about 30° to 40°, the affected tubers persist in a dry rot condition.

The organism is carried into the next season by infected tubers. When such tubers are planted, the fungus invades the shoots on which form the spores that infect the foliage. Another source of infection is the blighted tubers.

The losses due to this disease are of two kinds, losses caused by foliage infection which leads to premature death of the plant and consequently a reduction in tuber yield, and those caused by tuber infection and loss through rotting of infected tubers in the field and stores (Robertson, 1991). In the hills, the losses may be as high as 90% in a susceptible variety. In the plains (irrigated crop) losses mainly depend on the time of disease appearance.

Following infection and tissue colonisation by *P. infestans*, the biochemical processes undergo a drastic change in the infected tissue. In incompatible interactions, chlorogenic acid increased more pronouncedly than compatible ones (Doke, 1985; Doke and Chai, 1985; Doke *et al*., 1982). Lignin deposition is higher in incompatible interactions. It is now believed that increase in phenylalanine ammonia lyase (PAL) activity and lignin deposition in early phase of infection are indications of a race-specific reaction of potato tuber tissue to *P. infestans*. Deposition of insoluble phenolic or lignin like compounds is mostly associated with non-race specific resistance (Ampomah and Friend, 1988) whereas scopolin accumulation in infected tubers is most likely an indication of compatible interaction.

P. infestans also has a profound effect on accumulation of sesquiterpenoid compounds in the necrotic tissue. Rishitin was the first such compound discovered in infected tissue, since then many more phytoalexins have been isolated but only Rishitin, lubimin and phyttuberin are main phytoalexins which play role in disease resistance.

2.1.3 Control:

Late blight is primarily a foliar disease but also badly infects the tubers. Therefore, its effective management entails reduction of both foliar and tuber infections. This cannot be achieved by employing any single method. Instead, the three way approach, *i.e.*, use of cultural and chemical methods and host resistance have to be used for an integrated control of the disease.

Cultural Practices: These methods are important in blight control and mainly aim at eliminating or reducing the initial inoculum load in seed tubers and other sources and to check the spread of the disease. Rotations should be planned to avoid early and late maturing (main) potato crops in adjacent fields. Cull piles of waste potatoes should be disposed off properly and not allowed to sprout. Covering with black plastic sheets or spraying with herbicides will kill sprouts. It is desirable to reduce the chance of introducing blight in seed tubers. Certified seed should have very low level of blight, but it is still advisable to allow seed tubers to sprout,

and reject tubers which do not sprout (Parry, 1990). In the plains, the disease normally appears with the onset of winter rains. Therefore, irrigation should be completely stopped during the rainy period (Bhattacharya *et al.*, 1990).

Chemicals: Copper salts were found highly effective and, therefore, used extensively during 1885-1934 (Schwinn and Margot, 1991) against late blight. Despite their limitation of being strictly protective and requiring repeated applications, they were able to reduce the infection pressure resulting in an overall increase in crop yield. Dithiocarbamates and 1,2-bisdithiocarbamates were proved to be highly effective against this disease and were immediately put to wide use. Another group of fungicide *viz.* cyanoacetamide-oxime (cymoxanil) was discovered in 1976 which had better fungicidal activity and strong acropetal movement thus exhibiting both residual as well as curative action against *P. infestans* (Schwinn and Margot, 1991). Since then a host of other systemic fungicides have been discovered with better results. Metalaxyl (phenylamides) has by far proved the most potent fungicide ever evolved against late blight and within a few years of its discovery, its use increased rapidly and it became the major fungicide used for the control of late blight in Ireland (Staub and Schwinn, 1980). The first application of systemic fungicides should occur before blight is seen *i.e.* when plants meet along the rows. Depending on the risk of infection, repeated applications are, recommended at intervals of 10-12 days, with a maximum of 5 applications per season. Most growers use a combination of protectant and systemic sprays, as use of systemic fungicides alone over a long period leads to the formation of fungicide resistant strains. It is common for systemics to be used during the first part of the season, and protectant towards the end.

Host Resistance: During the early period of potato breeding, general resistance or field resistance was the only type of resistance available to breeders (Umaesus *et al.*, 1983). Although wild species like *S. demissum* were put to use for exploiting their blight resistance potential immediately after the Irish Potato Famine, still it was only in the beginning of this century that *S. demissum* was crossed with *S. tuberosum* and their progeny used in the resistance breeding programme in U.K. and Germany (Black, 1971). Later on several wild species like *S. phureja*, *S. andreanum* and *S. edinense* were exploited for their late blight resistance potential but *S. demissum* has been so far the most important source of resistance. In the beginning *S. demissum* derived resistance brought success as it provided immunity to the disease. However, the success was short lived as new compatible physiological races of *P. infestans* appeared almost simultaneously (Schick, 1932) making the use of race-specific resistance infructuous yet breeding for race specific resistance continued until the end of the 1960. Slowly, the attention was shifted to breeding for general field resistance. This kind of resistance does not exist any directional selection pressure on the fungus hence worked very well so far. There is hardly any increase in aggressiveness of *P. infestans* in association with the cultivars having a general resistance.

Field resistance is polygenically controlled and therefore difficult to handle in a back crossing programme as most of the gene flow only to a low proportion of progeny and that too disappear during the back crossing process (Ross, 1986). Besides several other *Solanum* sp., *viz. S. bulbocastanum,* and *S. versuosum,* also

possess high degree of field resistance and have already become part of the breeding strategies in some selected laboratories (Lardeo, 1989, Sharma *et al*., 1982). The current level of field resistance in *S. tuberosum* can be strengthened by combining this resistance with partial resistance from related *Solanum* species. (Behnke, 1980; Hermsen, 1980; Hermsen and Taylor, 1979). Alternatively, non-host resistance from related species can be transferred to *S. tuberosum* (Colon and Budding, 1988).

2.2 Early Blight (target spot)

Early blight of potato is caused by *Alternaria solani* Sorauer (Ellis and Martin). In India it is the most common and destructive disease of this crop and can cause upto 40% loss in yield when severe.

2.2.1 Symptoms:

Dark brown circular to oval leaf spots are formed, which frequently contain concentric brown rings giving rise to 'bull eye' or target board appearance. Mature lesions may be limited by leaf veins (Fig. 2). Lesions occur first and most abuntantly on the lower, senescent leaves which often become yellow. Dark brown to black lesions develop on infected stems. Symptoms may be confused with late blight but normally *Alternaria* occurs earlier in the season and there is no downy fungal growth associated with lesions.

Fig.2.: Early blight lesions on Potato leaf (left) caused by *Alternaria solani*

2.2.2. Disease Cycle:

The mycelium of the fungus remains viable in dry infected leaves for a year or more. Conidia have also been found to retain viability for 17 months at room temperature. Mycelium and conidia can thus survive in the soil on diseased plant debris to cause primary infection in the next years crop. Contamination of tubers with conidia or mycelium is another source of primary inoculum. Infection on lower leaves first takes place through conidia formed in soil. Secondary spread of the disease occurs through conidia developed on primary spots. These conidia are disseminated by wind, water and insects. Infection occurs, as a rule, through the stomata but direct penetration may also take place.

The disease becomes serious when the season begins with abundant moisture or frequent rains followed by warm and dry weather which are unfavourable for the host and help rapid disease development. Weaker plants are more susceptible to attack than plants with good vigour. Periods of continued drought check the spread. Incubation period varies from 48-72 hrs.

2.2.3. Control:

Since the disease is soil borne, crop rotation and field sanitation are essential for an effective control. However, crop rotation may not be a practical suggestion in many regions. Dead haulms should be raked together and burnt immediately after harvest. Timely spraying of fungicides, is, at present the best method of protection against the disease. The spraying should be started early, about a month after planting and should be continued throughout the period of plant growth at intervals of 10-21 days. Important fungicides that have been recommended for control of the disease are Dithane M-45 (0.2%), Blitox-50 (0.25%), Difolatan, Daconil, Brestan, Antracol and Captan.

2.3 Wart Disease of Potato

It is caused by fungus *Synchytrium endobioticum* (Schilberszky) Percival. It was first described in 1895 from Hungry. It is a serious disease of potatoes in temperate climates such as Europe, North America, Mountainous areas of South America as well as South Africa (Lapwood and Hide, 1971; O'Brien, 1976). In India the disease was first reported by Ganguly (1953) from Darjeeling and countinues to be endemic to that area.

2.3.1. Symptoms:

The principle symptom is the presence of rough, warty outgrowths or protuberences on tubers, stolons and stems of potato, also occasionally occurring on leaves and flowers. They never occur on roots. The warts are soft, spongy and more or less spherical. Their color resembles that of the host tissue. Above ground symptoms, when they occur, consists of yellowish-green cauliflower like outgrowths, often no above-ground symptoms are visible. Morphologically the wart consists of distorted, proliferated, branched structures grown together in to a mass of hyperplastic tissue.

2.3.2. Disease Cycle:

The fungus overwinters as resting spores in the soil or on the surface of seed tubers. Upon germination, motile zoospores are released which swim in soil moisture, encyst and penetrate the tuber surface particularly in the region of meristematic tissue. Tuber cells are then stimulated to multiply and warts begin to form. There may be further cycles of infection within the growing season as sporangia are produced in infected tissue, which releases more infective zoospores. Towards the end of the season zoospores fuse in pairs, resulting in the formation of resting spores which are released into the soil when the warted tissue rots. Such resting spores may remain viable for 30 years.

2.3.3. Control:

It is difficult to control the disease once it has been introduced in a field. The introduction of the disease in a field or locality can be effectively checked by practising quarantine *i.e.* prevention of entry of diseased material into healthy areas. Periodic surveys are required to monitor viability of the pathogen in soil and efficiency of the quarantine measures. Various experiments have been conducted to control the disease by soil treatment. Long crop rotations help to minimize losses and removal of diseased plants should be helpful in reducing the buildup of inoculum. The use of soil fungicides to eradicate the wart fungus is helpful but costly (Hodgron *et al*. 1974; O'Brien and Rich, 1976). However, disease resistance is the only effective measure to control the wart disease. The resistance of such cultivars has proved to be long lasting due mainly to the low dispersal capability of the pathogen, any new race of the fungus being restricted by lack of mobility to that area of soil where they were produced (Jones, 1987).

2.4   Stem Canker and Black Scurf

The disease is caused by *Rhizoctonia solani* Kuhn. The perfect basidial stage is *Thanatephorus cucumeris* (Frank) Donk.. The disease is of world wide occurrence and in India it affects potato tubers wherever the crop is grown. Major damage to yield of the crop is through the stem canker phase. The black scurf causes qualitative damages as it decreases the markebility of the tubers both for table purpose as well as seed.

2.4.1. Symptoms:

On haulm (stem canker), symptoms first appear on underground stems as brown cankers which may girdle the stems that emerge. Severe cankers can result in the formation of aerial tubers and cause rolling and wilting of foliage. Another characteristic symptom of stem cankers is the formation of a white powdery collar, again girdling the stem, just above ground level (Fig. 3). This is seen most frequently during humid early summer weather.

On tubers (black scurf) black or dark brown sclerotia develop on the surface of mature tubers. These may occur individually or aggregate to form large patches.

Fig.3.: *Rhizoctonia solani* sclerotia on surface of Potato tuber.

They are loosely attached to the skin and can easily be scratched off. Blemishing is the only damage to tubers, but when infected seed tubers are in humid stores, the sclerotia become active and the resulting mycelium infects sprouts killing their tips and roots. Young stems are weakened by the lesions and easily broken, and in severe attacks some plants may not emerge. Slightly attacked stems emerge and seem normal, but below ground lesions expand to form dry sunken cankers which may girdle the stem base and split longitudinally exposing internal stem tissues. Stolons are also attacked. Later in the growing season, the uppermost leaves on affected stems roll upwards and inwards, not unlike primary leaf roll, but the leaves are less stiff and the symptoms may disappear. The perfect stage of the fungus, which is produced in cool moist weather, appears as a whitish gray powdery film on the base of the stem, the lowermost petioles and branches near soil level (Fig. 4).

Fig. 4.: *Thanaetephorus cucumeris,* perfect stage of *Rhizoctonia solani* on Potato stem.

2.4.2. Disease Cycle:

The fungus overwinters on potato tubers as black sclerotia of variable size or mycelium on seed tubers, in the soil or on debris. Mycelium infects potato stem or emerging sprouts in spring and disease appears to be most severe in dry light soils, particularly when conditions are cold at the time of planting. Spores may be produced on the white 'collor' stage, but the disease usually spreads underground from plant to plant by mycelial growth. Tubers may become infected at any time during growth and sclerotia are formed towards the end of the season.

Damage to crops varies and it is most serious when developing sprouts are attacked, although some varieties may almost completely recover later. Injury to stolons interferes with tuber formation and infected stolons produce few large tubers, also the infected stolons are replaced later or sometimes branch, so increasing the number of small tubers. Although the total yield of tuber is unlikely to be greatly affected.

2.4.3. Control:

Since there is a risk of disease when infected seed tubers are planted, using healthy seed may decrease the chance of heavy infection and may prevent introducing the pathogen into soils. Besides, it is a polyphagus fungus with large host range and can survive through soil as well as seed over long periods. Successful management of the disease depends on proper management of the soil, the crop and treatment of the seed. Singh (1968) and Singh *et al*. (1972) had reported good control of the disease and high increase in yield of clean tubers by amending the soil at least 3 weeks before planting with neem or margosa cake (23quintala/ha) or with wood saw dust (25q/ha) followed by application of 120 kg nitrogen per hectare at the time of planting In soil, the fungus can be suppressed through the application of fungicides or the activity of its antagonists such as *Trichoderma* spp. Fungicides such as benomyl, carboxin, pencycuron, thiabendazole (Virgin-Callerus *et al*. 2000), are effective for the control of the disease. Biocontrol agents like *T. harzianum* (Mishra *et al*. 2000), *T. viride* (Arora, 1999), *Rhizoctonia* (Tsror *et al.* 2001) *Bacillus subtilis* and others have been identified to be effective against *R. solani*.

2.5 Powdery Scab

It is caused by fungus *Spongospora subterrannea* (Wallr.) Lagerheim. It is primarily a disease of cool climates (Hines, 1976). It has been reported from Africa, Asia, Australia, Europe, North America and South America (Miller and Pollard, 1976; Walker, 1969).

2.5.1. Symptoms

These are initially seen as small, slightly raised spots under the surface of the skin. The skin then breaks away leaving a ragged edge and a mass of brown powdery spore balls which distinguishes these primary symptoms from those of common scab caused by *Streptomyces scabies*. A more serious phase of powdery scab may develop, especially in wet soils. Tubers become deformed and wart-like growths may develop on tubers and, unlike wart disease, on roots as well (Fig. 5).

Fig.5.: Powdery scab, caused by *Spongospora subterranea*

2.5.2. Disease Cycle:

The pathogen overwinters as spore balls in soil and on the surface of seed tubers. These germinate to form motile zoospores which swim in soil moisture and invade root hairs, epidermal cells, lenticels or eyes, or penetrate through wounds in the tuber. Plasmodia are formed in tissue and may stimulate the tuber to produce a protective layer of cork which bounds the plasmodia. Secondary zoospores are formed from the plasmodia which spread the disease deeper into tissue and can also initiate infections. This is the most destructive phase of the disease. Spore balls are also formed which contaminate soil and can remain viable for 6 years (Parry, 1990).

The disease is seasonal and generally confined to fields with a history of the problem, when it occurs mild disease attacks reduce marketability of the crop and severe cankerous powdery scab often results in rejection of the crop.

2.5.3. Control:

Cultural control of the disease requires the practice of long rotations, improvements in drainage and destruction of infected tubers. Diseased tubers should not be fed to stock as manure will be contaminated with viable spore balls. Certified seed tubers should be grown. Highly resistant varieties are the cheapest and best solution of this problem. In screening of germ plasm cultures of *S. tuberosum* (CP 1742, 8-7), *S. tuberosum* x *S. microdontum* (BRB/A-24) and a polyploid of *S. tuberosum* (JHT/A-1214) had shown no infection under natural and artificial inoculation conditions (Bhattacharya *et al*. 1985). However, none of the commercial varieties under cultivation in India are resistant. The exotic varieties- Panther, Patron and

Red Skin are highly resistant to powdery scab. Attempts have been made at Central Potato Research Institute (CPRI), Shimla to develop commercial varieties suitable for local conditions by using the hybrids of *S. andigena* x *S. tuberosum* as one parent and local varieties like Kufri Jyoti and Kufri Chandramukhi as other parent (Singh, 1995).

2.6 Pink Rot

It is caused by fungus *Phytophthora erythroseptica* Pethybr (Goss, 1949; Hodgson *et al*. 1974; O'Brien and Rich, 1976).

2.6.1. Symptoms:
The fungus attacks tubers and roots of the plant. Affected tubers tend to have dark lenticels and a rubbery texture. If squeezed, they exude a watery fluid and it is common for particles of soil to stick to diseased tubers. When tubers are cut open, affected tissue is initially an off-white colour. However, within 30 minutes, the tissue turns a salmon-pink colour and eventually purple-brown or black. Diseased tubers often smell of vinegar. Pink rot is primarily a disease of potato tubers. However, plants may become wilted late in the season, and aerial tubers may form on the stems. Infected roots and stems turn brown or black, resembling blackleg (Hooker, 1981; O'Brien and Rich, 1976).

2.6.2. Disease Cycle:
Pink rot is favored by warm, wet summers and excessive irrigation. The pathogen survives for many years in infested soil as oospores. Upon germination, mycelium or zoospores infect all underground parts of the potato plant. Rotten infected daughter tubers release more oospores into soil. The disease can not spread to healthy undamaged tubers in store, but in poor stores wounded potatoes may be susceptible to infection. The fungus is favored by high soil moisture and temperature of about 23°C.

2.6.3. Control:
Only healthy seed potatoes should be planted on land where potatoes have not been grown recently. Wet, poorly drained soils and excessive irrigation should be avoided. Tubers should be handled very carefully during the harvesting process so as to prevent wounding. Only healthy tubers should be stored under relatively cool, dry conditions (Rich, 1983).

2.7 Silver Scurf

Helminthosporium solani Dur. & Mont. (Conners, 1967; Western, 1971) causes the prevalent superficial blemish of potato tubers, silver scurf.

2.7.1. Symptoms:

The symptoms of this disease are confined to the tubers. The most obvious symptom is development of a smooth, gray, leathery skin, especially near the heel end. Symptoms can develop prior to harvest or in storage. They are most conspicuous when tubers are wet at which time they exhibit a silvery sheen hence the name 'silver scurf'. Severely affected tubers shrivel and shrink due to loss of moisture. The disease long considered to be of minor importance can be a cause of blemish of wore tubers washed before sale (Western, 1971).

2.7.2. Disease Cycle:

Infection probably originates from infected seed tubers or from tubers left in the soil from a previous crop. The fungus penetrates through lenticels and skin periderm and remains within the cork cells, where the hyaline mycelium eventually turns brown. It does not penetrate into the tuber tissue, but air pockets develop between the affected cork layers producing the silvery appearance. Some periderm tissue slough off, the tubers lose water and eventually shrivel. The temperature range for the fungus growth is 2-31°C with an optimum between 21°C and 27°C. Cultivars vary in susceptibility, but none atre known to be resistant.

The disease is very wide spread but because of its superficial nature, it is relatively unimportant in most crops. However, it can reduce the markebility of a crop because of its deterimental effect on the appearance of diseased tubers and loss of water in storage.

2.7.3. Control:

Potatoes should be harvested as soon as they are mature. If they remain in moist soil, severity of the disease will increase. Most infections originate during storage, especially in warm and moist conditions when *H. solani* sporulates on affected skin, producing a sooty black appearance. Spread of disease within stores can be prevented by storing at temperatures below 3°C and at relative humidity below 90 % and on seed tubers by chemical disinfection soon after lifting. Soil treatment with pentachloronitrobenzene may be beneficial (Wright, 1968).

2.8 Watery Wound Rot (leak)

Pythium ultimum (*Pythium debaryanum*) causes the watery wound rot of potato tubers. Some tubers are infected in most seasons but serious losses occur only in crops harvested immature in warm weather or transported or stored in bulk in warm and humid conditions.

2.8.1. Symptoms:

As the name implies, affected tubers develop a watery soft rot and moisture oozes or 'leaks' from them. They may or may not show external symptoms. The flesh of infected tubers is granular, very watery and the colour varies from light yellow to

shades of brown or black. The decayed area is usually delineated by a dark brown or black line. Rotted tissue may become pulpy and develop cavities.

2.8.2. Disease Cycle:
The fungus lives in soil and enters tubers only through abrasions or wounds, so infection usually occurs at harvest, cut seed tubers can also be attacked and cause 'blanks' or weak points. The skin around a lesion becomes dark and moist and as the rot advances, affected tissues shrink causing the skin to stretch, the skin breaks when touched and oozes a watery material. At a temperature of about 22°C whole tubers may be rotted in a few days, but spread can be arrested by keeping tubers cool. When cut partially, diseased newly dug tubers may show a black zone between diseased and healthy tissues. The diseased tissue, smelling faintly alcoholic turns gray on exposure to air, then brown and finally black, sometimes with splashes of pink. Rotted tissue may become pulpy, develop cavities and smell fleshy and sometimes the whole inner tuber tissues may be rotten and hollow, leaving only tissue outside the vascular ring intact. Fungal oospores in soil, in the field or adhering to harvested tubers germinate and penetrate tubers through damaged tissue. More infection sites may be initiated in store in infected tubers by the production of sporangia. Rot may then progress quickly, especially at temperatures around 21°c. Tubers left to rest in the field will further contaminate soil with oospore.

2.8.3. Control
The disease is only occasionally serious but it may be the initiator of more serious problems, such as soft rot, which occurs in potato stores. The effects of the disease can be minimised by limiting the harvesting and handling damage to immature crops and by keeping tubers cool. They should not be shipped immediately after harvest (Blodgett and Rich, 1950). Diseased tubers should be removed from the field and buried (preferably burned) to prevent the build up of the soil population.

2.9 Gangerene

Gangerene is a storage disease of potato, caused by *Phoma exigua* Desm. var. *foveata* (Foister) Boerema).

2.9.1 Symptoms:
The disease is not normally seen until potatoes have been stored for at least a month. First symptoms of the disease are small dark round or oval depressions in the wounds, eyes or lenticels. Lesions gradually enlarge giving characteristic 'thumb-mark' depressions covered by smooth darkened skin. They can vary from 6-50 mm in size with well defined edges. Sometimes dark pycnidia in lesions are also visible. When affected tubers are cut open, large cavities lined with a white fungal mycelium may be seen inside.

2.9.2 Disease Cycle:

The fungus overwinters in soil either in the field or in potato stores. Seed tubers and groundkeepers also serve as overwintering sources of inoculum. Infection of potato haulm tissue and developing tubers occurs during the season, but goes largely unnoticed. Pycnidia may be formed on senescent tissue at the end of the season and spores released can be washed through the soil and contaminate tubers. High temperatures in late summer tend to reduce the disease and low temperature storage (2-6°C) which inhibits the healing process in potatoes encourage disease development.

2.9.3. Control:

Disease resistance is available in currently grown varieties, although no particular variety is outstanding in this respect. Varieties differ in their susceptibility to most storage diseases, including gangerene. The most important control measure is careful handling at harvest time to prevent bruising, and storage at 15ºC for 10 days to promote rapid healing of wounds.

Chemical treatment of tubers after harvest with benomyl thiabendazole (TBZ) or fuberidazole controlled gangrene in storage as effectively as an organo-mercurial dip. A combined application of 1% benomyl and 1% captafol was more effective than either one above (Copeland and Logan, 1975).

2.10 Dry Rot

It is caused by various *Fusarium* spp. such as *F. coeruleum* (Lib.) Sacc.. *F. eumartii* (Carpenter) Synder & Hans, *F. oxysporum* Schlechtend and *F. sulphureum* Schlechtendahl.

2.10.1. Symptoms:

Symptoms of dry rot usually occur in tubers which have been stored for a number of weeks. Initially, small brown areas may be visible on the tuber surface. The surface of infected tubers is wrinkled and may be sunken, and the rolled tissue may turn brown, gray or black. Cavities frequently develop in affected tubers, which may become more or less filled with yellow, pink or red *Fusarium* molds. After prolonged storage it is common for gray, white, blue, black, purple, or pink spore masses to develop on the surface of infected tubers.

2.10.2. Disease Cycle:

Fusarium species survive in the soil as resting spores (chlamydospores). Seed tubers may also be contaminated with chlamydospores. Infection usually occurs as a result of damage of tubers and progress well in poorly ventilated humid stores.

In the early days of mechanisation of potato harvesting, there was a dramatic increase in dry rot in stores, because of the damage done by unrefined potato

harvestors. This is much less of a problem now. However, some seed crops still have problems with dry rot, mainly because of the extra handling and storage involved in their production. Some older varieties are very susceptible to the disease.

2.10.3. Control:
Cultural control of dry rot involves minimising damage at harvesting and during subsequent handling. Well-ventilated cool stores will also help to reduce the disease.

Chemical control, particularly for seed tubers, may be worthwhile. Spraying tubers with 1200 ppm thiabendazole or benomyl control the disease (Leach, 1978; Leach and Nelson, 1975). Although resistance to thiabendazole in Fusaria has been observed (Hanson, 1996). Control of *Fusarium* through biocontrol agents such as *Trichoderma* spp. (Pinzon *et al.* 1999), *Pseudomonas aeruginosa* (Gupta *et al*. 1999) have been found to be effective.

2.11 Skin Spot

It is caused by fungus *Polyscytalum pustulans*.

2.11.1. Symptoms:
The fungus can develop on all underground parts of the potato plant, giving rise to general browning. Light brown lesions develop on stems, stolons and roots. However, infections of the tuber during storage are most significant. After several months in store, small discrete spots 0.5-2.0 mm in diameter with raised centers occur on the tuber surface. Skin spot may develop over the entire tuber surface, including the eyes.

2.11.2. Disease Cycle:
The fungus overwinters in soil as microsclerotia and in dry soil in potato stores. Diseased seed tubers, however, are the main initial source of inoculum. Infection spreads to underground parts of the plant throughout the season and is usually concentrated around the eyes. Affected tubers are generally symptomless at harvest and only after storage do spots develop. Damp conditions in potato stores can result in further infections of tuber *via* air-borne conidia.

The disease may be important in two respects. Firstly, the cosmetic quality of a crop is reduced as a result of skin spot infection, and secondly, colonisation of potato eyes can reduce sprout numbers resulting in non-emergence of plants.

2.11.3. Control:
Cultural control may be worthwhile in tubers for seed. Fungicides should be applied as soon as possible after lifting.

2.12 Wilt of Potato

It is caused by *Verticillium albo-atrum* which induces wilting of the tops and vascular dislocations of the stems, tubers and roots – symptoms similar to those associated with other wilt-inciting fungi. Verticillium wilt on potato was reported in Europe and America early in the twentieth century. It occurs in the seed potato areas in the New England, North Central and North Western States (Parry, 1980)

2.12.1. Symptoms:
Early symptoms involve epinasty and wilting or 'flagging' of the leaves. Gradually the leaves turn dull green, then yellow (chlorotic) and finally brown (necrotic). Symptoms progress upward until the entire stem is affected.

2.12.2. Disease Cycle:
The wilt fungus is harbored in the tubers and persists in the soil. If conditions are favourable, wilt free soil can be infested by wilt-infected seed potatoes. Attempts of getting wilt free seed tubers by cutting off the discolored stem end of infected tubers have met with failure because fungus hyphae may penetrate beyond the discolored section of the tuber.

2.12.3. Control:
Infected and/ or infested seed potatoes should be avoided. As the disease is spread from place to place via infested soil adhering to the surface of seed tubers should be decontaminated with the effective chemical. Captan and metiram are among the recommended and approved fungicides. Liquid treatments are superior to dusts because they remove more of the infested soil (Cetas, 1970; Cole *et al*. 1972; Easton *et al*. 1972). One kilogram of active ingradient suspended or dissolved in 500 litres water should be effective. Crop rotation is an important cultural practice. Potatoes should be grown in rotation with cereals, grasses, legumes or other nonsusceptible crops. There are many resistant cultivars available like Abnaki (Akeley *et al*. 1971), Cariboo (Maurer *et al.* 1968), Cascade (Hoyman, 1970), Nooksack (Hoyman and Holland, 1974), Raritan (Campbell and Young, 1970), Targhee (Pavek *et al*. 1973), Batoche, Belrus, Campbell, Campbell 13, Tobique, CF7353-1 (Murphy *et al.* 1982).

2.13 Charcoal Rot

It is caused by *Macrophomina phaseolina*. The disease probably occurs in all tropical and subtropical countries where potatoes are grown. The disease is of minor importance on potato, but may become severe following a period of unusually warm, wet weather. It can also affect wounded tubers during storage (Chupp and Sherf, 1960).

2.13.1. Symptoms:

Most infection centres are at the lenticels but some tubers may show stem end rot. A soft, dark-colored shallow rot develops on the lower stem area, resembling black leg. Secondary organisms frequently follow primary infection by *M. phaseolina*. The fungus enters through infected stolons. Around the lenticels black areas appear and slowly spread all over the tuber surface. Inside the tuber also the flesh shows blackening. After heavy rains the whole tuber may decay as a result of invasion by soil saprophytes which cause soft rot.

2.13.2. Disease Cycle:

Under normal conditions, *Macrophomina* is a weakly parasitic soil fungus. It has a wide host range (Ranga Rao and Mukerji,1971, 1972; Ranga Rao *et al,* 1973). It attacks potato plants when the soil is warmer and wetter than optimum for good potato production. Fungal growth and sclerotial development are rare at 10°C or below. The optimum temperature for growth and infection is about 30°C. Poor plant nutrition favours development of the disease. Wounds predispose tubers to infection. The fungus overwinters as sclerotia in soil and plant debris, and can also live from season to season in perennial weeds and other crop plants.

2.13.3. Control:

Proper nutrition, drainage and crop rotation should reduce the incidence of this disease. It was observed by Thirumalachar and O' Brien (1977), that treatment of whole tubers with a strain of *B. subtilis* reduced the frequency of charcoal rot at harvest.

3.   Bacterial Diseases

3.1   Brown Rot (Bacterial Wilt)

It is caused by bacterium *Ralstonia solanacearum* (Smith) Yabunchi *et al*. Earlier the pathogen was called as *Pseudomonas solanacearum* (Smith). The disease occurs in tropical, subtropical and warm climates and devastating nature of the disease has changed cropping patterns in some parts of countries like India, Indonesia and Peru (Gufran and Chakravarti, 1960; French, 1986; Machmut, 1986). Earlier, the disease was not considered to be a limiting factor in potato cultivation in many parts of India (Butler, 1918; Mann and Nagpurkar, 1920), but later surveys revealed negligible level of wilt incidence (Bhinde, 1959; Patel *et al*., 1952). Later surveys (AICPIP, 1989-90, Gadewar *et al*., 1991; Shekhawat *et al*., 1978,) revealed prevalence of the disease in sixteen states of India.

3.1.1. Symptoms

Sudden wilting of plant is the characteristic of bacterial wilt. Potato harvest from diseased plants may not show any external symptoms. However, in general, infected tubers indicate brown discoloration in the vascular region, a slight pressure makes bacterial ooze out of the vascular region and in advanced stages often bacterial masses ooze out from tubers.

Brown rot symptoms on potato haulms are manifested as wilting, stunting and yellowing of foliage. Transient wilting during the day, with recovery at night, often leads to a permanent wilt and death soon follows. In young potato plants it may be possible to see brown colonized xylem vessels through the epidermis. Cut stems freely ooze grayish-white bacterial slime from xylem vessels. However, the first symptom of brown rot in tubers is a browning of the vascular ring. If squeezed, pale yellow bacterial ooze is visible. This may contaminate the surface of the tuber, especially around the stem and eyes, resulting in soil adhering to the tuber surface.

3.1.2. Disease Cycle:

The primary initial source of inoculum is mildly, or latently infected seed tubers, however, weed species may also harbour the pathogen during winter. Invasion ofvthe host plant occurs primarily through wounds. The disease is particularly severe in wetter climates and it rarely occurs in areas where mean soil temperature is below 15°C.

Assessment of economic losses are difficult as the crop plants wilt before tuberization and produce from infested fields continue to rot in storage. Reported damages are often based on the wilting in the fields. In India, losses recorded are up to 55% in Kumaon hills (Hari Kishore and Pushkarnath, 1963), 0.33-40% in Maharashtra (Paharia, 1963), 20-25% in Hyderabad (Nath *et al*., 1958) and over 75% in some localities of Karnataka (Gadewar *et al*., 1991).

3.1.3. Control:

Under Indian conditions, Shekawat *et al*. (1988a,b) observed that application of bleaching powder @ 12kg/ha mixed with fertilizer or soil drenching before or after first earthing up reduce the bacterial wilt incidence by 80% and increased potato yield. As the pathogen is susceptible to high temperature, raising the soil temperature by covering soil with polythene film has been found to be effective in reducing wilt.

The bacterial wilt pathogen forms a heterogeneous group on the basis of its host range, its soil borne nature and host parasite-interactions, which are greatly affected by environmental factors. Hence, its control practices ought to be directed towards reducing the inoculum in the field and avoiding introduction of fresh inoculum. Growing the crop, when the environmental conditions are unfavourable for the pathogen and developing resistant varieties or by biological means such as use of the avirulent strains of the pathogens and/or rhizobacteria also help in reducing the disease incidence. Unfortunately, resistant varieties are resistant to only a few of many strains of the pathogen. Rhizobacteria and avirulent forms of

the pathogen can be a potential tool to manage the disease. The mechanism involves exclusion of pathogen by colonisation, antagonism and induced resistance by avirulent forms of *R. solanacearum* and release of bacteriophages by lysogenic strains of *P. solanacearum* (Chen and Echandi, 1984). Bacterial species like *P. fluorescens*, *Bacillus* spp., *B. polymyxa* and actinomycetes have been found to reduce the wilt development and incidence (Gadewar and Shekhawat, 1988b, Shekhawat *et al*. 1992). Avirulent mutants of *P. solanacearum* were found to protect potato plants from virulent strains (McLaughlin and Sequeira, 1988).

Crop rotation with wheat, maize, sunn hemp and vegetables like cabbage, onion and other reduced the wilt incidence to the extent of 94% (Gadewar *et al*. 1991; Shekhawat *et al*. 1980b. 1992a). By this successful reduction in wilt incidence has been obtained in both hills and in Indian plains.

3.2 Blackleg and Soft rot

Blackleg, caused by *Erwinia carotovora* subsp. *atroseptica* (Jones) Bergy *et al*.is recognised by an inky-black lesion on the base of the stem which is a primary distinguishing character from similar soft rot caused by bacteria.

3.2.1. Symptoms:
Infected plants develop characteristic symptoms. The leaves turn yellow and roll upward, when plants are relatively small. Plants tend to stand upright. At first the underground portion of the stem turns black, but as the disease progresses, the inky black color advances up the stem for several inches. The stem may become slimy. Severely affected plants wilt and die. The disease also progresses downward through the stolons and into the tubers. During wet conditions, either in the field during a rainy summer or in a poorly ventilated, warm humid store, the tuber rot will develop quickly, causing total distingeration of tissue. Alternatively, it is possible in dry conditions for rot to be confined to a relatively small area around the heel end of the tuber.

3.2.2. Disease Cycle:
The soft rot of tubers, which may occur in the field if soil is moist and temperature is high or during transit and storage, the tubers are transformed partly or totally, slowly or quickly, into a soft decayed pulpy mass. The mass is held together only by the corky epidermis which can not be attacked by the parasite. When a soft tuber is cut open, the colourless putrid mass turns a pinky red on exposure to air rapidly becoming brownish red to brown (Singh, 1995). In very wet weather, the inky black lesions at the base of the stalk may spread to most of the plant. The bacteria enter the new tubers through the stolons of a blackleg stalk and invade the vascular elements, as well as other tissues of the tubers. Affected tubers show soft rot, involving the entire tuber. Under less favourable conditions the decay is arrested so that only the tissues in the center of the tubers are disintegrated.

3.2.3. Control:

In very moist weather, bacteria in the soil invade freshly cut or poorly healed seed pieces – a possible explanation for the more general appearance of blackleg or wet than in dry season in some places. Because cuts, bruises and other injuries permit the entrance of rot-inciting organisms, tubers should be handled carefully to avoid bruising (Agrios, 1997). Storage should be provided with favourable temperatures and humidity for healing over (or suberizing) injured tissues. Removing from the seed potatoes, all tubers showing rot and storing cut seed potatoes immediately after cutting at about 70°F and 80% humidity to favour adequate healing of the cut surface have been effective control measure in some places. In the USA, experiments have shown that the tuber rot and black leg can be controlled by treating the seed pieces with antibiotics (Bonde, 1955; Robinson and Hurst, 1956). Application of stable bleaching powder before planting is helpful as it increases emergence and yield (Parashar and Sindhan, 1988; Karwasra and Parashar, 1998). Control of soft rot by microbial antagonists like strains of *Pseudomonas fluorescence* and *P. putida* have shown promise in the field (Abdelghafor and Abdekayed, 1997; Kartelein *et al*. 1999), but no commercial products were available most likely owing to the difficulty of making commercially stable formations. Sharga and Lyon (1998) identified a *Bacillus subtilis* isolate BS 107 that controlled ECC and the closely related *E. carotova* subsp. *atroseptica* (ECA). The potential for commercial product development is greater with bacilli owing to the presence of endospore. They provide considerable resistance to mortality caused by environmental fluctuations.

3.3 Ring Rot

Ring rot is caused by *Corynbacterium sepedonicum* (Spieck. & Kotth.) Skapt & Burkh. It is one of the most contagious and most feared diseases of potatoes, especially among seed potato growers.

3.3.1. Symptoms:

It is recognised by wilt of the foliage and rot of the vascular ring of the tubers. Chlorosis or yellowing and marginal browning and wilting of the leaves are symptoms. In the tubers the disease is detected by a light-yellow discoloration of the vascular elements which break down and exude a cherry bacterial and cellular ooze when a tuber is squeezed. It is common for secondary bacterial pathogens to invade tubers colonised by *C. sepedonicum* and tubers are then quickly reduced to semi-liquid state.

3.3.2. Disease Cycle:

Ring rot apparently is not harbored in the soil but infected tubers overwintering in the soil may develop infected volunteer plants which may serve as source of infection. The disease is spread from infested to healthy tubers by the seed cutting knife, planting machines, grading equipment and contaminated hands, gloves, bags, baskets, barrels and bins that have come in contact with diseased potatoes.

3.3.3. Control:

Control involves prevention, sanitation and use of resistant varieties. It is primarily the responsibility of seed-potato growers, who should propagate and maintain their own seed potatoes. Potatoes that are free from ring rot can be kept so if no infected seed potatoes are introduced from other source

3.4. Common Scab

Common scab is caused by an actinomycete *Streptomyces scabies* (Thaxter) Lambert & Loria.

3.4.1. Symptoms:

It is recognised by slightly raised spots or lesions of rough, corky tissue on the tuber. The entire surface is involved. Scab lesions spoil the looks of the tuber and cause waste in peeling and reduction in grade. There are two types of lesions formed on the tubers: i) the shallow and ii) the deep scab. In shallow scab the affected tubers show superficial roughened areas, sometimes raised above, and often slightly below the plane of the healthy skin. The lesions consist of corky tissue which arise from abnormal proliferation of the cells of the tuber periderm due to attack of the pathogen.

In deep- pitted scab the lesions measure 1-3mm or more in depth and are darker than the lesions in shallow scab. They also are corky and may join together so that the entire tuber surface becomes affected. The deep pitted lesions are either extension of the shallow lesions, combined effect of the scab organism and some chewing insects, or due to some specific strains of the scab organism

3.4.2. Disease Cycle:

The organism is widely distributed throughout soil and penetration of the developing tubers usually occurs via lenticels. Some of the important conditions which influence the development of scab are soil acidity, moisture, temperature and aeration. A soil reaction below pH 5.2 is unfavourable for most of the common scab races, although some strains are said to cause infection below pH 5. Common scab develops at a wide range of temperatures, 50° to 85° but it thrives best at about 70°. Relatively high soil moisture tends to check the disease in some localities, but in other districts high moisture may favour scab.

3.4.3. Control:

Treatment of seed tubers with disinfectants kill the scab fungus on the tuber, but it fails to control the disease if the treated tubers are planted in scab infested soil. Therefore, scab-free seed potatoes should be planted in scab free soil. Usually potato scab can be controlled by maintaining the soil pH between 5.0 and 5.3 (Hooker, 1957). The use of 300 to 500 pounds of sulphur on area reduces the severity of scab in some soils. The use of ammonium sulphate (Huber, 1980) and single super phosphate (Grewal *et al*. 1988) in fertilizer that increase soil acidity

may inhibit the disease to some extent. An application of pentachloronitrobenzene (Brassicol) at the rate of 20-30 kg/ha reduces the disease.

The most promising method involves the development of scab-resistant varieties. Studies in Europe have disclosed that Jubel, Hindenburg, Ostragis, and Anaica are scab-resistant. It is caused by several species of *Streptomyces* commonly found in soils worldwide. *S. scabies* is considered to be the most common species causing scab. While resistant cultivars are available, many susceptible cultivars are used because of these specific market characteristics. Management of this disease can be achieved in part by management of soil moisture during early tuber formation, maintenance of low soil pH, use of green or animal manures, and the use of seed treatment fungicides to reduce seed borne inoculum. Maintenance of high soil moisture during early tuber development allows for higher populations of antagonistic bacteria and lower populations of actinomycetes on the tuber surface as compared to dry land (Adam and Lapwood, 1978). They demonstrated that the effect of moisture was not on *S. scabies* since it infected equally well in inoculated dry or wet sterile soils. It is likely that the control achieved through the use of green or animal manures is due to increased microbial activity.

4.   Potato Viruses

Potatoes are infected by almost three dozen viruses belonging to different virus groups. Their important characteristics like shape, size, genome group, variability, transmission mode, symptoms *etc.* are listed in Table 2. Some of these viruses are mainly associated with and are dependent on potatoes for perpetuation and spread while others can perpetuate and spread through other hosts as well. The major types of viral diseases, caused by these viruses, may be categorised as Mosaics (Khurana, 1992; Khurana and Garg, 1992) leafroll necrotic spots/necrosis *etc*. Mosaics and leafroll are important due to their ubiquitous distribution and tuber yield reduction. Potato viruses causing mosaic symptoms include potato viruses X,S,A.,Y.V.M. Andean Potato latent virus (APZV), Andean Potato mottle virus (APMV), Potato aucuba mosaic virus (PAMV), tobacco rattle virus (TRV), Tobacco streak virus (TSV), Potato mop top virus (PMTV), Potato yellow dwarf virus (PYDV), white leafroll is caused by potato leafroll virus (PLRV) and necrotic leaf spots and stem/petiole necrosis by a tomato spotted wilt group virus (Tospovirus) (Khurana *et al*., 1989). Besides these important viruses potatoes are also readily infected by potato spindle tubers viroid (PSTVd).

TABLE 2
Characteristics of Virus Diseases of Potato

Virus (sp.)	Disease Caused	Particle Size/nm (RNA Strand) Shape	Mode of Transmission	Host Range
Potato virus A (PVA)	Supermild Mosaic	730 x 13 (1)	Aphids NP	Solanaceae
Potato Leaf Roll Virus (PLRV)	Leafroll	24-26 ison (1)	Aphids P	Restricted
Potato Virus M (PVM)	Leafrolling Mosaic (Paracrinscle)	650 x 12 (1) flexuous	Contact	Restricted
Potato Virus S (PVS)	Latent	650 x 12 (1) flexuous	Contact	Restricted
Potato Virus X (PVX)	Latent/Mild Mosaic	515 x 13 (1)	Contact	Wide
Potato Virus Y (PVY)	Severe/Ruqose Mosaic	730 x 11 (1)	Aphids	Wide
Potato Virus T (PVT)	Mild/Transient Mosaic	640 x 12 (1)	Pollen/Seed	Wide
Anden Potato Latent Virus (APTV)	Severe Mosaic	28, ison (1)	Contact Beetle	Wide
Alfalfa Moaic Virus (AMV)	Calico/ Yellow Mosaic	58, 204 x 18 (3)	Aphids NP	Wide
Potato Aucuba Mosac Virus (PAMV)	Aucutx Mosaic	580 x 12 (1) flexuous	Contact/Aphids	Restricted
Potato Mop Top Virus (PMTV)	(i) Mop Top (ii) TMV-PMTV	300 x 18 (1) Rods	Injury/Fungi	Wide
TSV (Ilar Virus)	Faint Mosaic	28, isom (4)		Wide
Tobacco Mosaic Virus (TMV)	Necrotic Mosaic	26, isom (1)	Fungi	Wide
TRVQ (Tobravirus)	Rings-Pot/Leaf Reduction & Spraing	45 x 90 180-210	Seed/nematodes	Extensive
Tomato Spotted Wilt Virus (TSWV)	Stem/Leaf Necrosis	80 (70-90) (1) Spheroidal	Thrips	Extensive
SALCV	Apical Leafcurl	17 (Triplets)**	White Flies	Restricted
Potato Spindle Tuber Viroid (PSTV)	Spindle Tuber Naked RNA (i)	Small, Circular Free	Contact/Seed	Wide

NP/P = Non Persistently/Persistently Aphid Borne
HC = Helper Component Required for Aphid Transmission
** = The Only DNA Virus Infecting Potatoes

Due to vegetative propagation, virus(es) infection having once taken place, goes on increasing in intensity and incidence with successive propagations resulting in uneconomical yield in 3-4 years. The viruses are difficult to control chemically, hence they are controlled

by an integrated schedule for viral management comprising different aspects such as use of virus-free stocks, exclusion of virus(es) and avoidance of vector(s) during the crop growth, vector(s) control, cultural process to minimise virus(es) contamination/speed, elimination of virus(es) through tissue culture and chemotherapy from valuable clones/cultivars, resistance breeding and incorporation of virus-genome derived resistance into desired varieties through genetic engineering (Khurana *et. al*., 1992).

4.1 Yield Losses

Potato tuber yield reduction rate due to virus infections is influenced by a variety of factors like the virus strain, host cultivars, current or secondary infection, time of current infection, proportion of plants infected, growth behaviour of the cultivar, climatic and edaphic conditions. Due to too many factors affecting tuber yields of virus-affected potato plants, precise estimation of tubers yield loss due to a particular virus is quite difficult under field conditions. It is further complicated by the occurrence of mixed virus infections which are very common under natural conditions. Generally, tubers yield losses are 5-15% when all the plants are secondarily infected with PVX and PVS; 15-30% for 100% secondary infection of PVYN, and 40 to 70% for infection with PLRV and PVYO (Arenz and Hunnius, 1959; Bonde *et al*., 1943; Borchardt *et al*., 1964). Further these figures only indicate the range of losses after experience in the Temperate Zone under normal climatic conditions. Adverse or extreme climatic conditions like high temperature and drought may result in higher losses.

4.2 Epidemiology

Potato viruses spread from plant to plant within a field and between fields through mechanical means as well as vectors PVX, PVS and PSTVd spread through cutting of tubers into small pieces at the time of planting and/or mechanical injury to tubers and sprouts and contact of infected foliage with the healthy workers contaminated clothes,

implements, farm machinery *etc*. No insect vector is known for PVX and PSTVd. However, PSTVd may be disseminated through the female of root knot nemotode, *Meliodogyne incognita* passively (Annonymous, 1992). Besides, PSTVd is also most readily seed transmitted. Many apterous forms of aphids have been shown to transmit PVY under experimental conditions. But apterous forms are important in plant to point spread of the virus in the same field whereas alatae are required for sitant spread of the virus(es). About 30 species or species group of alatae aphids may transmit PVY (deBokse and Pirone, 1990; Harrington and Gibron, 1989).

4.3 Management

Potato viruses adopt different strategies for their spread and perpetuation. Consequently, they have to be contained or managed through a package of strategies, *viz*., exclusion, heat therapy, healthy seed production, vector control, resistance breeding and incorporation of virus resistance through genetic engineering (Fig. 5).

4.3.1 Exclusion

Some of the potato viruses occur only in certain specified areas and not the others. Thus, APMV, APLV, PVT and PVR normally occur in the Andean region. Elsewhere they have reached only through germplasm which originated from the Andes. Besides, these viruses (except APMV, PVR) are also true seed transmitted. Many of these have several well-characterized strians (Beemster and Rozendaal, 1972) and new virus are still being identified (Fribourg and Nakashuma, 1984) Traditionally virus escapes were bulked uo for the production of 'virus free' seed. During multiplication, plants showing virus symptoms were rouged out. Now, virus-free seed is generally bulked up in geographically vector free areas, *e.g.* in Ireland and Scotland or vector populations are monitored and seed tubers lifted, depending on the infection pressure, aphid (vector) flights and age of crops as in Holland (Hiddema, 1972).

4.3.2. Heat Theraphy and Meristem Tip Culture

Since the 1960's heat therapy and meristem tip culture have been used to eliminate virus from potato (Mellor and Stace-Smith, 1977; Quak, 1977). The sensitivity of detection methods has also been increased by the introduction of immunological procedures, principally, the Enzyme-linked immunosorbent assay (Clark and Adams, 1977).

Virus Reservoirs
(Weed, Infected crops)

Herbicides

Vector

Oils   Insecticides
Virus free stock → Young crop → Mature crop

Heat treatment
Meristem culture

Virus Infected stock

Virus infected plants
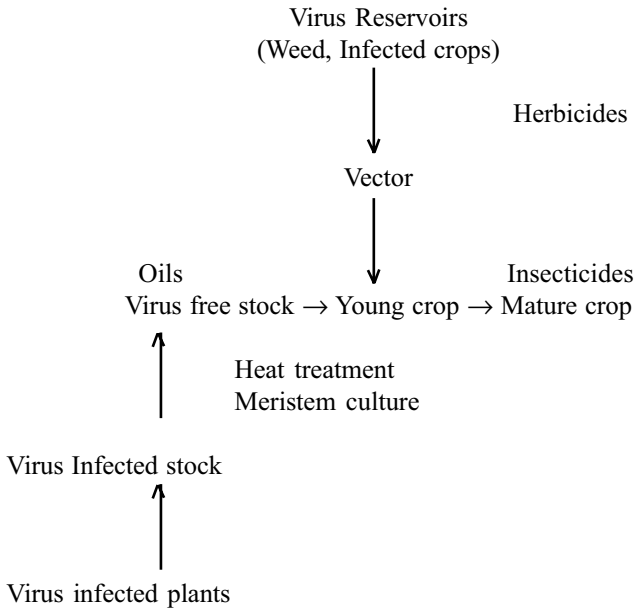
Fig. 5. : Strategies for virus Control

Independently, heat therapy and meristem culture have made an important contribution to virus elimination in potato certification schemes. Heat therapy, however, is empirical in that the temperature regime depends on the virus starch/ host variety combination (Hollings and Stone, 1968). Occasionally, heat stable straths of potato viruses have been reported (Mellow and Stace-Smith, 1970, 1971). According to Krylova *et al.* (1973), failure to eliminate viruses in meristem culture, in some cases, may be due to the invasiveness of certain potato viruses. The limitations of heat therapy and meristem culture as individual procedure may be at least, partially overcome by their combination (MacDonald, 1973). Application of elevated temperatures during *in vitro* culture has been suggested for the elimination of invasive viruses (Walkey and Cooper, 1975).

Elimination of potato viruses through chemo- and thermo-therapy without the meristem tip culture was tried by Griffith *et al.* (1990). *In vitro* potato plantlets were exposed to an alternating 18h light/36°C and 8h dark/31°C cycles and ribovarin (20 mg/litre) therapy for 4 weeks. They were further propagated *via* nodal cuttings at room temperature without exposure to ribavirin. Ribavirin alone or in combination with heat not only reduced the virus titres (10 and 60 fold) but also freed more number of viruses including PVY and PLRV (Conrad, 1991).

Survival of meristem tips, less than 0.5mm, required for virus elimination is generally very limited. This problem is overcome by first growing the infected plant for several weeks at 36°C before taking the meristem tips which could be much larger in this case (Stace-Smith, 1985). Chemo-therapy using antimetabolites and antiviral chemicals *viz.* Virazole (ribavirin) (Dhingra *et al.* 1987, Walker, 1980) has also been reported to improve the recovery of virus-free plantlets from

larger meristem sections. Subsequently, regeneration of potato plants from meristem tips and stem explants of virus-infected potatoes resulted in the elimination of PMV, PVS, PVX and PVM (Cassells and Long, 1982; Klein and Livingstone, 1982, 1983a,b).

4.3.3 Vector Control

Many insecticides are effective against the spread of PLRV whereas virtually none is effective against the non-persistently aphid-transmitted viruses like PVY and PVA (Sigvald, 1984; Stelznee, 1950; Van Hoof, 1980). This is due to very smart periods required for acquisition and transmission of PVY by aphids. Only pyrethroids with quick knock down effect on the vector have been reported to show some promise in the control of PVY (Perrin and Gibson, 1985). Besides pyrethroids mineral oils have been reported to reduce the spread of non-persistently aphid transmitted viruses (Bradley *et al*., 1966). 50-90% of prevention of PVY spread with mineral oil sprays has been reported (Schepers *et al*., 1984). Nonetheless, use of mineral oils for PVY control has not become popular probably due to high material and labour costs (Schepers *et al*., 1978).

4.3.4. Healthy Seed Production:

Availability of virus-free or healthy planting stocks is the first requirement for an economical potato cultivation. Improvements in the virus detection technology coupled with rapid multiplication *in vitro* have resulted in better health standard of potato crop and increased tubes yield (Dodds and Horton, 1990; Khurana and Garg, 1992; Khurana *et al.* 1989, 1996). Generally, virus free stocks are multiplied in high hilly tracks swept by strong wind currents reducing the vector pressure. Alternatively, they are multiplied in regions having climate suitable for potato crop production but lacking the aphid vector(s) or having their population below and certain critical level during the crop growth. In India, virus-free potato stocks are referred to as breeders seeds which are multiplied in high hills above 2000m insummer and in Indo-Gangetic plains during autumn (Sept./Oct. to Dec./Jan.) when the vector activity is non to negligible.

Multiplication of virus free stocks *in vitro* has gained importance in many countries with tropical climate (Dodds and Horton, 1990). The pool of virus free stocks of the specified multiplication serve as the basic or nucleus seed from which certified seed is raised by further multiplication in either state owned farms or by the registered grower under direct supervision of the qualified persons.

5.   Non-parasitic Diseases of Potato

5.1 Black Heart

It is a very common disease, which may occur in the field as well as during storage. It occurs in the field when the soil temperature rises

above 32.2°C during growth and maturity of tubers. It occurs in transit when the temperature inside the carrying vans rises, for sometime above 32°C and during storage, when the tubers are stored in poorly ventilated rooms in closely packed conditions.

It is due primarily to asphyxiation. Dark gray to purplish-inky black discolouration occurs in the central tissue of the tuber, which may extend to the surface of the tuber also in advances stages. The affected tissue dry out and separate thus forming small or large cavities (hollow heart condition) in later stages.

In poorly ventilated rooms even low respiration by tubers uses up the available supply of oxygen which results in discolouration and distintegration of cells due to adverse enzymic action which continues after the supply of oxygen has diminished.

It can usually be controlled by proper ventilation and temperature control of storage sheds and freight cars. The temperature in heated cars should not be allowed to rise above 16°C to 21°C. Tubers should be removed from hot, dry soils as soon as the vines die, and should not be allowed to remain on hot, dry soils after digging (O' Brien and Rich, 1976).

5.2. Nutritional disorders

Nitrogen deficiency is common in potatoes and is expressed by light green to yellowish-green colour of the foliage whereas deficiency of phosphorus causes stunting of the plants and rusty brown flecks on the tubers. In potash deficiency, the plants are stunted and leaves are dark green. In magnesium deficiency, lower leaves show chlorosis on the margins and at the tips. It increases and the entire interveinal area may turn yellow (Singh, 1995, *see* Chatterjee and Dube *this volume*).

5.3. Freezing Injury

Potato tubers are damaged by very low temperatures in cold storage as well as in the field. At temperature, just above freezing point, the tubers become sweet in taste due to conversion of starch into sugar. However, this sweet taste disappear when the tubers are kept at 20°C for sometime. In the fields, low temperature injury occurs, when there is frost or snowfall. Internal necrosis is the main symptom. There are three types of necrosis depending upon the time for which the tubers

are exposed to low temperature. In ring necrosis, there is discolouration in the region of the vascular ring and more commonly at the stem end. In net necrosis. Finer vascular elements of the inner phloem, scattered throughout the tuber within the vascular ring are blackened. In blotch necrosis, there are irregular areas of different sizes showing opaque to black discolouration. It is the result of maximum exposure to low temperature.

Potatoes should be stored at temperatures above 20°C to prevent the conversion of starch to sugar, resulting in a sweet potato. This will also serve as a protection from mahogany browning, tuber necrosis, or freezing and collapse. If tubers develop a sweet taste, they should be stored at about 15°C for 1 or 2 weeks. The sugar may be utilized during respiration.

Storage bins, trucks and freight cars should be insulated and heated in cold weather. Potatoes should be exposed to below freezing temperatures.

6.   Biotechnology of potato improvement

Potato is the first major food crop where biotechnology has been successfully applied (Bajaj, 1986). The high productivity, good nutritional quality and high amenability of potatoes to genetic improvement through biotechnology make the crop more suitable for genetic manipulation through these techniques. In fact, most of the frontier technologies like tissue culture, genetic engineering and molecular breeding are routinely used for improvement of potato. In developed countries, potato has already been identified as the target crop species for improvement by genetic engineering. Some of the traits in which success has already been achieved or work is going on are resistance to viruses, fungal diseases, insects, herbicides, a biotic stresses, improvement in quality and pharmaceutical possibilities.

6.1 Resistance to fungal diseases

One of the important biotic threat to potato is posed by fungal diseases, most important among them is the late blight caused by *Phytophthora*

infestans. Efforts to develop transgenics against late blight are still permature. Chitinase and glucanase, which hydrolyze two major cell wall components of fungi, will not be useful against *P. infestans* because cell wall of this particular pathogen contains cellulose instead of chitin. Moreover, cellulose-degrading enzyme cannot be used for control of plant. Therefore, alternative strategies based on host's defence mechanism were developed to control this disease. The active defence of plants against fungal attack involves generation of reactive oxygen species like H_2O_2. Enhancement of *in vivo* H_2O_2 synthesis can therefore confer resistance to fungal attack. Wu *et al*. (1995) developed transgenic potato plants expressing a glucose oxidase gene cloned from *Aspergillus niger*. Glucose oxidase converts glucose to gluconic acid and H_2O_2. The transgenic plants thus obtained showed improved late blight resistance.

Similarly, osmotin gene encoding a close of pathogenesis related proteins has also been transferred into commercial potato cultivars for improved resistance to *P. infestans*. Because of membrane disrupting properties of osmotin, it inhibits hyphal growth *in vitro* and cause sporangial lysis of *P. infestans*. Transgenic potatoes have been produced and delayed disease symptoms were observed on detached leaves of these plants after inoculating with *P. infestans*.

In recent years impressive work has been done to understand molecular mechanism of race-specific resistance against *P. infestans* in potato. Several major resistance genes have been tagged with molecular markers. Once the basic mechanism underlying vertical resistance against late blight pathogen in potato is understood, several strategies can be designed for production of late blight resistant transgenic potatoes. Late blight resistance also involves a hyper sensitive response which is a manifestation of programmed cell death (Dangl *et al*., 1996).

6.2 Resistance to Bacterial Diseases

Bacterial diseases like soft rot, brown rot and common scab are responsible for appreciable yield loss in potato. Total crop loss from brown rot and soft rot can be as high as 30-100% during cultivation and 2-6 months storage periods, particularly in tropical countries where

potato is kept in country stores. Classical breeding for resistance to bacterial diseases is so far not successful mainly due to lack of resistant genes in potato germplasm, which leaves only use of antibiotics and agronomic manipulations to control bacterial diseases. Genetics of antibiotic production is a complicated phenomenon and not a single attempt has so far been made to transfer antibiotic producing genes into crop plants. However, a class of simple antimicrobial peptides produced by vertebrates, arthropods as well as by some plants as a response to invasion by different biotic agents, have recently attracted attention of biologists. The most extensively investigated class of such antimicrobial peptides comes from insects. The dispausing pupae of giant silk worm moth (*Hyalophora cercropia*) synthesis more than 15 new types of proteins in their haemolymph, when challenged by heat killed pathogen or non-pathogenic bacteria. Among these, cecropins, attacins and lysozymes have been found to have potent bactericides properties. These peptides possess a broad spectrum of antibacterial activity against both gram positive and gram negative bacteria. In collaboration with the Louisiana state University, USA, the International Potato Centre, Lima, Peru has evaluated efficacy of these lytic peptides for the control of bacterial soft rot and brown rot in potato. Gene construct encoding most of these lytic peptides are now available. In fact, genes encoding SB-37 and Shiva-1 (two analogues of cecropin B) have been introduced into potato. Transgenic plants when inoculated with virulent *Ralstonia solanacearam* showed delayed symptom appearance, reduced disease severity and less plant mortality. Disease response of such transgenic plants were comparable to that of a field resistant cultivar Cruza-148 (Montanelli *et al*., 1995).

6.3 Resistance to Potato Viruses

As mentioned earlier potato is infected by more than 20 viruses. Potato viruses are not only important because of the yield losses they cause, but also because most of the viruses are transmitted through tubers. The simplest transgenic strategy to contain viral damage in potato is through coat protein (CP) mediated resistance. CP-mediated resistance is multi component type of resistance, exhibiting resistance to virus infection, multiplication, expression and spread.

Resistance to plant viruses can also be achieved by transferring pathogen-derived sequences that are not translated into any protein products inside the plant. Highly resistant transgenic potato lines have been developed by transferring such untranslatable viral DNA sequences (Goodwin *et al*., 1996). For virus suppression through this strategy it is necessary that the viral genome have some sequence identify to the transgene (English *et al.*, 1996). The mechanism is referred to as homology-dependent gene silencing and it has a tremendous potential in developing virus resistance transgenic plants.

6.4 Tolerance to Herbicides

Use of herbicides to control menace of weeds is an integral part of modern agriculture. Weeds compete with the crop and may reduce yield upto 76% (Neild and Proctor, 1962; Chitsaz and Nelson, 1983). Application of herbicides is expensive, moreover, many cultivars are sensitive to them. Some of the commonly used herbicides such as chloracetamide and as-triazine can cause stunting and reduced yields (Weler *et al*. 1979; Freeman, 1982). Thus the best strategy is to develop herbicide-resistant/ tolerant plants. The industry could see double benefits from this technology. Once a herbicide resistant crop is produced by an industrial house, it can look forward to control the seed market of the transgenic cultivar as well as that of the herbicide. Herbicide resistant commercial cultivars of several crops, such as cotton, corn, soybean, flax and carola, have been released during 1995-97 (Birch, 1997). Glyphosate is a potent broad spectrum non-selective herbicide which inhibits production of an enzyme 5-enolpyruvylshikimic acid 3-phosphate synthase (EPSP Synthatase) involved in biosynthesis of aromatic amino acids. The gene overproducing EPSP synthase has been isolated from Petunia and introduced into tomato at the Monsanto Co., making tomato plants tolerant to glyphosate herbicides. Tomato plants overproducing EPSP synthetase have also been engineered to contain multiple copies of EPSP synthatase gene, which has been introduced into potato. Transgenic potato plants over expressing the enzyme (40 times more than normal plants) were demonstrated to be resistant to glyphosate..

Resistance to bromoxynil, another potent herbicide, which inhibits photosystem II. is achieved by incorporating a gene whose product

deactivates this herbicide. The gene for this deactivating principle (nitrilase) was isolated from the soil bacterium *Klebsiella ozaenae*. The gene was placed under the control of tobacco rbcs (small subunit of ribulose biphosphate carboxylase/promoter and T-DNA octopine synthase gene (OCS) terminator and was then transferred into potato. The transgenic plants were resistant to the herbicide bromoxynil.

6.5 Tolerance to Abiotic Stresses

As a consequence of wide spread cultivation throughout the globe, potato is often exposed to extreme weather that may seriously compromise its productivity.

Exposure of growing plants to water deficit caused by either drought, salinity or freezing is manifested as an assay of changes in cellular processes (Bray, 1993). The clue to transgenic strategy for drought tolerance come from analysis of plants that grow under desert (xerophytes) or extreme saline (halophytes) conditions. These plants survive water stress by accumulating sugar alcohols like mannitol, sorbitol and mycoinsitol that may act as osmolytes and help in osmoregulation. These sugars also helps in osmoprotection, serving as scavengers of active oxygen generated during water stress. Transgenic tobacco expressing bacterial mt/P gene that encodes an enzyme for mannitol synthesis showed tolerance to high salinity. The same gene may be introduced into potato for improvement of drought and salt tolerance (Shekhawat *et al.*, 1997).

In potato freezing injury is manifested in water-soaked appearance of the foliage due to leakage of electrolytes through damaged cell membrane into the intercellular spaces. The damaged tissue cannot recover from this shock and as a result the affected foliage often dies. The major effect of chilling on plant cell is manifested in loss of membrane permeability. Certain leaf colonizing bacteria act as nucleus for ice formation and frosting on leaves. These are called ice nucleation active (INA) bacteria. *Pseudomonas syringae* is one such bacterium. "Ice minus" mutants of this bacterium were produced by deletion of ice nucleation gene. Application of these mutants to potato seed pieces at planting and subsequent spraying on plants after emergence, provided protection to potato plants from frost injury.

Certain tuber defects *e.g.* internal black spot formation develops, while potatoes are stored in piles. Black spots are caused by oxidation of tuber phenolics like chlorogenic acids to melanins by polyphenol oxidase (PPO). In field trails, transgenic potatoes in which activity of PPO was down regulated by antisense technology showed less enzymic browning and internal black spot formation (De Bath *et al.,* 1996). Expression of alcohol dehydrogenase (ADH) and/or aldolase (ALD) genes in potato is being investigated to control hypoxic stress related injury (less availability of oxygen) in potato tubers.

6.6. High Nutrition Potato

Potato is a highly nutritious, mild flavoured, easy to blend food that has many possibilities for 'building in' desired nutrients. It is a rich source of carbohydrates which yield energy. Being low in fat and high in moisture (75-80%) the preparations which allow for decrease in moisture and increase in fact cause an increase in energy content. It can be combined easily and effectively with a number of foods, and it contains adequate well balanced proteins (2-3% fresh weight) as well as a number of vitamins (carotene in yellow potatoes, vitamin C, thiamine, riboflavin and niacin) and minerals (low sodium, high potassium, utilizable iron), thus the possibility of 'building in' nutrients using biotechnological methods is tremendous.

The nitrogen fraction of potatoes contains 40-60% free amino acids. The increase in amino acid using resistance to make alterations in synthetic pathways offer possibilities for 'building in' essential amino acids leading to a favourable nutritional balance. Potato cell lines with varying amounts of tryptophan (Carlson and Widhoem, 1978), proline (Van Swaai *et al*., 1985) and tryosine as well as phenyl alanine (Jacobson *et al*., 1986) have been developed.

Genetic engineering offers a noval approach for modifying the essential amino acid composition of plant proteins, and can thus improve their nutritive quality. Brazil nut, (*Bertholettia excelsa* H.B.K.) produces a methionine rich protein which contains 19% methionine and 8% cysteine. Transgenic plants of potato cultivars Russet Burbank and Atlantic have been produced that express BN 25 gene of Brazil nut. The expression of this gene was, however, 8-fold

lower in transgenic tubers in comparison to leaves. Tuber expression of this gene is being improved by utilizing tuber specific promoter such as patatin. At the Louisiana State University, USA, a synthetic gene producing 80% essential amino acids was prepared and named High Essential Amino Acid Encoding (HEAAE) gene. This gene was transferred to two potato clones K-2 and K-7. Protein analysis of transgenic plants showed that HEAAE protein comprised 0.02-0.35% of the total plant protein and there was about 1.1% increase in essential amino acids (Shekhawat *et al*. 1997).

It has become possible with the use of biotechnology to build in specific nutrients, flavours, tastes, shapes and other organoleptic qualities that improve potato as a food and its nutrients. Biotechnology can also be used to obtain single cell proteins using potato starch through immobilized biocatalyst technology (Knorr and Sinskey, 1985). Both in terms of built-in nutrients and products biotechnology offers a variety of choices.

Genetic engineering may allow production of Cereal quality starch in potato. Starch can be chemically fractioned into two types of glucan polymers amylose (30%) and amylopectin (70%). Starch is synthesised in leaves during the day time from photosynthetically fixed carbon and is mobilized to storage organs at night. The biosynthetic steps required for starch biosynthesis involve three enzymes. ADP glucose pyrophosphorylase (ADPGPPase), Starch synthase (SS) and starch branching and debranching enzymes. ADPGPPase catalyses the synthesis of ADP-glucose from glucose-1-phosphate, which is the precursor for synthesis of both types of starch. Therefore, the over expression of APPGPPase would produce tubers with higher starch content. Tranasgenic potato expressing *E. coli* glg C16 gene encoding the bacterial APPGPPase, showed remarkably high starch content (60% more than normal) in tubers. Starch synthase, branching and debranching enzymes determine the quality of starch (Smith *et al*., 1997). The amylose content of starch is regulated by an enzyme granule bound starch synthase I (GBSS I). Starch with reduced level of amylose contents has been produced from transgenic potato in which GBSSI activity has been reduced through antisense technology.

The degree of branching of amylopectin is determined by the activities of starch branching and debranching enzymes. Expression

of Starch branching enzyme gene (glg B) of *E. coli* increased degree of branching in amylopectins by 25% in potato (Kortstee *et al.*, 1996).

As potato is easy to grow and can generate considerable biomass within a short period of time, preliminary research has been carried out to determine whether transgenic potato can be exploited for the production of commercial proteins and biochemicals. Transgenic potato has been developed that can synthesize fructans in tubers (Van der Meer *et al*., 1994; Rober *et al.*, 1996). Similarly a disacharide, trehalose is produced in transgenic potato tubers expressing ots A and ots B genes of *E. coli* (Goddijn *et al.*, 1997). Similarly, transgenic potato can be used for production of pharmaceuticals, monoclonal antibodies, functional recombinant antibody fragment called plantibodies, and polyhydroxy-butyrate polymer which can be used to make a biodegradable plastic (Poirier *et al*., 1992).

7.   Conclusion

The potato, being a short term crop, can quickly yield energy and protein, thus potato would require an improvement in the protein quantity and quality as well as extension education for better utilization. In the developed countries potato takes the form of a low-fat, low-sodium food. The multiple use of potato also indicate the variation in the traits considered desirable for each purpose, for instance, potato to be used as feed for pigs and those used for fresh fries or for fuel energy need different built-in qualities. Thus, in the 21st century, different kinds of potatoes are to be developed to suit the dietary habits, meet the nutritional deficiencies of specific populations and be appealing enough to the concerned consumers.

Some of the qualities of potatoes which appeal to the consumer are their skin colour and texture, shape, taste, cooking quality and the price. The plant breeder is primarily concerned about disease resistance and yield. A nutritionist would like it to be energy packed and proteinaceous. For a post-harvest technologist, it should be easy and economical to store and transport.

The future potato is thus a variety of materials and challenges the creative ability of the scientist and the desires of the consumers.

Biotechnology, through cell culture and in vitro genetic manipulations is quite competent to meet the challenge of specific demands in order to 'tailor' the potato to match the need of the people. Due to amenability to biotechnological tools and its importance as a major world food crop, potato has been extensively used for biotechnological manipulations. These advances are only the beginning of a "second green revolution" and coupled with conventional breeding will lead to "low input" agriculture by providing farmers with "tailored" seeds which can protect themselves against biotic and abiotic stresses and require less fertilizers. This would also reduce the amount of hazardous chemicals in the environment and foodstuff. The main aim of all these developments is to make potato cultivation more efficient, more economical and environmentally safer.

The biotechnological innovations have profound applications in other areas too, such as healthcare, development of industrial products and environmental management.

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8

Seed-borne Fungal Diseases of Onion, and their Control

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ABSTRACT: *Aspergillus niger*, *Botrytis aclada* and *Fusarium oxysporum* f. sp. *cepae* are relevant seed-borne fungi of onion (*Allium cepa* L.) and are known as causal agents of black mould, neck rot and basal rot diseases, respectively. These pathogens can be transmitted from infected seeds to seedlings, sets or bulbs. They eventually kill the entire plant through degradation of the tissues. Different principles are suggested for their control. *Botrytis squamosa*, *Cladosporium allii-cepae* and *Stemphylium vesicarium*, which cause several lesion on the onion leaves, can also occur on seeds but they are not able to demonstrate disease transmission to the plant parts. The role of other seed-borne fungi (namely, *Alternaria alternata*, *Aspergillus alutaceaus*, *A. flavus*, *Beauveria bassiana*, *Cladosporium cladosporioides*, *Colletotrichum dematium*, *Curvularia lunata*, *Drechslera australiensis*, *Humicola fuscoatra*, *Stemphylium botryosum*, *Trichoderma harzianum*, *T. pseudokoningii* and *Trichothecium roseum*) on development of onion diseases is not known.

1. Introduction

Onion (*Allium cepa* L.) seeds have been found in Egyptian tombs built in 3200 B.C. and some authorities believe the onion may have been one of the first vegetables domesticated by humans. Today onions are important crop worldwide and China ranks first in total production of dry onion with 12 438 000 tons and, India, the United States and Turkey follow it with 4 900 000 tons, 3 060 000 tons and 2 200 000 tons, respectively (FAO, 2001). The unique flavor and odor of onions have made them an excellent food source. The recent popularity of a health conscious world for salad bars has increased their agricultural importance.

Three system of planting are employed in onion production: direct seeding, the use of sets and transplanting. Most commercial production

of bulb onion is done using direct seeding, though the more expensive option of transplanting and sets may be used when timing is a critical factor. Thus, management of seed diseases is very important for onion production and yield. However, since onion seeds are black colored it is not possible to see the symptoms of the disease unless the seeds are incubated.

There are only two reviews concerning the *Fusarium* basal rot and purple blotch diseases in onion (Aveling, 1998; Cramer, 2000), although there are a lot of research papers on onion diseases. Some research papers report the occurrence of fungi on seeds while others focus on the transmission of pathogens to seedling, sets or bulbs or on control methods. This review summarizes the present knowledge on the seed-borne fungi which can be transmitted to plant parts, diseases caused by them and epidemiology, the interactions between these pathogens and onion diseases, and their control possibilities. It also attempts to review the available literature on other possible seed-borne fungi.

2.   Major Seed-Borne Pathogens

2.1. *Aspergillus niger* Van Tieghem

A. niger causes black mould on the onion bulbs. It occurs on both colored and white onions in the field, during transit or during storage and has been reported in the United States, the United Kingdom, Australia, Spain, Chile, Japan, India, Nigeria, Sudan and Turkey (Sumner, 1995c; Köycü and Özer, 1997). The disease was also observed on 10 % of the total dry onion shipments inspected in the New York market during 1972-1984 (Ceponis *et al*., 1986). This pathogen attacks many fruits and vegetables through wounds or during ripening (Sumner, 1995c).

2.1.1.   Description
The conidia are black, spherical, irregularly roughened, and are borne in chains. Conidiophores arise from long, broad, thick-walled, mostly brownish, sometimes branched foot cells. The conidiophore axis swells to form a vesicle on which prophialides are formed. Phialides (sterigmates) are borne in clusters from the prophialides. Spore clusters can be seen without magnification.

2.1.2. Symtoms and epidemiology

The symptoms of black mould, caused by *Aspergillus niger* begin to appear at germination stage of seeds, continue until the storage and also in the store. The pathogen reduces seed germination, seedling emergence and vigour (Gupta *et al*., 1984; Tanaka, 1991; Hayden and Maude, 1992; Özer and Köycü, 1997; El-Nagerabi and Ahmed, 2001). *A. niger* generally causes significant reduction in germination of seeds, in severe cases, root and shoots can not develop because of pre-emergence damping-off of seeds (Özer and Köycü, 1997). However the pathogen causes post-emergence damping-off (Gupta and Mehra, 1984). The development of symptoms is closely related to temperature. *A. niger* has negative effects on seedling development at 30 ºC and 35 ºC, but fails to develop on seedlings grown at 13 ºC and 15 ºC (Hayden and Maude, 1992). Any visual symptom is not observed on set bulbs developing from contaminated seeds. However visual symptoms can be seen on mature bulbs in the fields and in store. *A. niger* firstly appear as small black spore masses under the outer dry scales of the bulb, spread as strips lying from the base to neck parts. When the outer covering (dry scale) is removed the spore masses are observed (Figs. 1,2) The fungus grows on the inner scales of the bulls in similar manner (Fig. 2). In severe instances spore masses cover all over the surface of the bulbs tissues.

Black mould may also be a problem in temperate areas, where the bulbs are dried at high temperatures before storage. In many developing countries, bulbs are stored in mud, straw huts or stack, which may leak during the rainy season. This conditions cause high humidity coinciding with optimal temperature for the growth and pathogenicity of *A. niger* (Thompson *et al*., 1972; Musa *et al.,* 1973; Maude and Burchill, 1988; Hayden *et al.,* 1994 a, Coskuntuna and Özer, 1997).
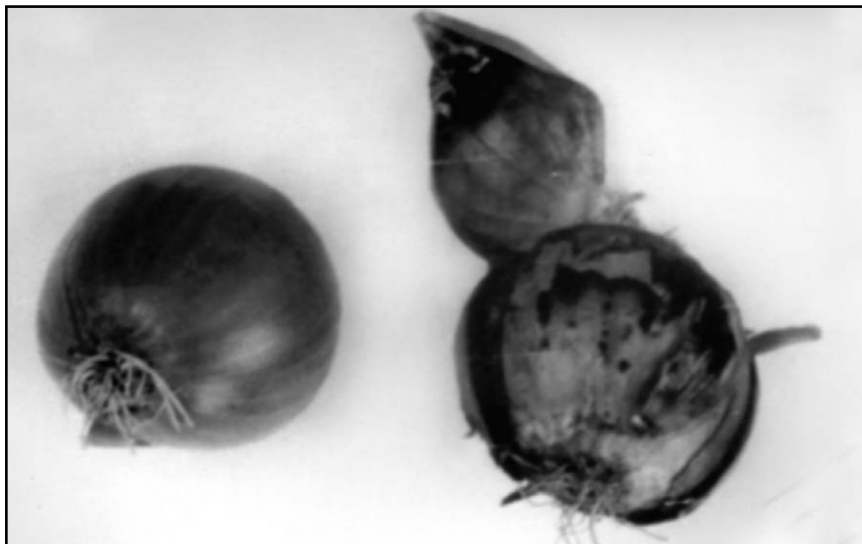
Fig. 1 : Outer dry scales infected by *Aspergillus niger* (left) and healthy scales (right).

Fig. 2 : Inner scales infected by *Aspergillus niger*.

Contaminated seeds are mainly responsible for the introduction of *A. niger* into the seedbed. The pathogen was found to be high on seeds produced in hot (dessert) climates (Hayden and Maude, 1992; Hayden *et al.,* 1994b). In addition, seeds produced in hot tropical regions such as Sudan, India and Yemen are more likely to be contaminated with fungus than those produced in temperate climates such as Holland and France. It was dominant species in all seed samples from the regions of Turkey (Köycü and Özer, 1997). The highest rate of contamination of seed coat by *A. niger* in Turkey was where the ambient temperature was 31.5 ℃ during the seed development in July. However *A. niger* was also isolated from all the seed parts (embryo, endosperm and seed coat) at high rates in two regions having the temperatures of 20 ℃ and 24 ℃ in July. The rate of virulence of *A. niger* depends on the different regions with different temperature ranges.

The pathogen can be transmitted from naturally contaminated seeds to seedlings (Hayden and Maude, 1992; Özer and Köycü, 1997). The base of plant is affected by spores from contaminated seeds during the emergence of cotyledon; the cotyledon elongates forming a looped structure which eventually breaks the surface of the ground, while the seed remains below ground level; *A. niger* may act as weak pathogen in this position as it sometimes develops on green tissue, it spreads to the dead tip of the cotyledon and then develops saprophytically on the cotyledonary leaves but does not invade green tissue at this point (Hayden and Maude, 1992). In addition, seedling infection is more severe when contaminated seeds with *A. niger* from tropical Sudan were used, than those of contaminated seeds from temperate United Kingdom. In contrary to this opinion, Sirois *et al.* (1998) showed that the isolates from the seeds of temperate region also caused high level of infection in seedling. Otherwise they suggested that *A. niger* appeared to have a systemic nature in its ability to colonize the onion seedling tissue.

Transmission of this pathogen from naturally contaminated seeds to set bulb is possible, without showing any symptom during the seedling development. When these sets are examined, *A. niger* can be isolated easily from the roots and bulb tissue (Köycü and Özer, 1997). It seems that the onion set is a source for the latent infection of this pathogen.

Hayden and Maude (1994 b) observed that *A. niger* could also be transmitted from contaminated seeds to stored bulbs. This fungus is also soil and airborne. It was determined that it could be transmitted from contaminated soil to seedlings and sets in temperate and hot climatic conditions (Hayden *et al*., 1994 b; Köycü and Özer, 1997; Özer and Köycü, 1997). In the tropical Sudan its incidence in the air increased progressively in onion crops during the growing season (Hayden *et al*., 1994b). Mechanical wounds during harvesting, packing and storage are the other transmission means of the pathogen.

Air contamination is important for the infection of seed stalks and flowers. *A. niger* can utilize the vulnerability of the flowers to penetrate the onion seed. When *A. niger* spores reach on the mature capsule prior to flowers opening, the possibility of seed infection is the highest. The pathogen has also the capability of saprophytic parasitation on the senescing onion flowers and systemic invasion to other parts of onion plant and seed, to maintain its survival and reproduction (Sirois and Lorbeer, 1998).

The optimum temperature for fungal growth is 28-34 °C; and growth is inhibited at 47 °C. Thus the disease is more common in hot climates (30-35 °C) or under warm storage conditions (24-30 °C). Spores germinate well at relative humidity of 80-86 %. Free moisture must be present on the onion for 6-12 hours for infection to occur (Hayden and Maude, 1992; Sumner, 1995c).

2.1.3. Host Parasite Interactions

The increase of oxalic acid in the bulbs after infection by *A. niger* is considered to be a significant factor in the rapid destruction of onion bulbs by pathogen (Tanaka and Nonaka, 1981). The components of onion bulb have the stimulatory effect on germination of *A. niger*. Onion scales contain glucose, mannose at the ratio 3.5:1, large amounts of glutamic acid, aspartic acid, alanine, leucine, threonine, arginine, histidine and potassium (Tanaka and Nonaka, 1983; Tanaka, 1991). *A. niger* has the ability to produce polygalacturonase (PG) enzyme and isoenzymes. PG isoenzymes are constitutively present in the spores of this pathogen obtained from naturally infested onion seeds and sets (Özer *et al*., 1999a). PG activity is optimal at 40 °C and pH 4.0. Presence of PGs in non-germinated conidia shows the possibility that these enzymes are involved in early stages of infection. *A. niger* produces exo-PG on onion seeds, although it has typical endo-PG activity *in vitro* conditions. PG enzyme and isoenzymes of this pathogen contribute to virulence during onion seed colonization (Özer *et al*., 1999a). Furthermore it was observed that the seeds of the cultivar Rossa Savonese from Italy, contained antifungal

fluorescence compounds which have important role on resistance to *A. niger* during the seed germination (Özer *et al.,* 1999b)

2.1.4. Control

Cultural practices include; thinning out of seedlings produced from seeds in the seedbed and in the field, thereby reducing spread of the pathogen within crop canopy; avoidance of continuous cropping of onions on the same site; removal and incineration of onion leaves from the field after harvest; minimum disturbance of the foliage is checked during the growth of the crop to prevent the release of *A. niger* conidia; regular ventilation of stores is done to maintain humidity levels at less than 80% (Hayden *et al.*, 1994a).

Any onion cultivar, at high resistant degree has not been considered. However, the cultivar Akgün 12 revealed tolerance to infections of pre- and post-emergence damping-off and set rot after seed infestation with *A. niger* in controlled pot experiments (Özer, 1998). Whereas, the seeds of the cultivar Rossa Savonese also exhibited the resistance to the pathogen during the germination (Özer *et al*., 1999b).

A. niger causes rapid and extensive tissue degradation. Its elimination from seeds is very difficult. Onion seeds should be treated with fungicides to help prevent seed rot and damping-off (Sumner, 1995d). Results of *in vitro* studies previously suggested that carbendazim (Qadri *et al*., 1982), benomyl, thiram, benomyl+thiram, prochloraz and tebuconazole (Özer and Köycü, 1998) were the best chemical products in controlling the pathogen. Among them benomyl and thiram were used as a treatment for reducing seed-borne *A. niger* of onion (Gupta *et al*., 1984; Hayden *et al*., 1994 c). It was reported that treatment of *A. niger* infested onion seeds with benomyl dust (1g ai/kg seed) or foliar spray of thiram (0.4g ai/ha) to plants grown from infested seeds under temperate (UK) conditions reduced the incidence of *A. niger* in the harvested crops. However, when seeds were naturally infested with this pathogen the treatment of benomyl+thiram to seed (2.5+2.5 g ai/kg seed) or soaking the seed in hot water (15 min at 60 °C) reduced the incidence of black mould on bulbs grown in the field soil of Sudan that had not previously been used for onion production. In addition, these treatments were less effective in crops produced in fields regularly used for onion production (Hayden *et al*., 1994c). Furthermore, it was suggested that prochloraz (0.90 cc ai/ kg seed) and thiram (1.35 g ai/kg seed) were the most effective chemicals for controlling *A. niger* infestations from seeds and soil respectively (Özer and Köycü, 1998).

In recent years, alternative compounds or treatments to pesticides were evaluated. El-Neshawy *et al*. (1999), reported that dip and spray treatments of seedling with a commercial product of *Trichoderma* (Promat) prevented *A. niger* infection on the bulbs. El-Nagarabi and Ahmed (2001) found that surface disinfection of onion seeds with 10 % garlic water extracts and sterile distilled water at 60 °C reduced seed infection, pre- and post-emergence damping off and this also enhanced the growth of seedlings in the field. Özer *et al*. (2002) found that incorporation of the stalks of sunflower, alfalfa and Hungarian vetch,

especially sunflower stalks – to soil after the harvest suppressed set rot by *A. niger* in naturally infested soil.

2.2. *Botrytis aclada* Fresen (syn.: *B. allii* Munn)

B. aclada is the causal agent of neck rot disease in onion. The pathogen has been considered as dominant species causing disease in the United Kingdom (Maude and Presly, 1988a,b), Germany (Bochow, 1981; Rudolph and Bräutigam, 1990), New Zeland (Stewart and Franicevic, 1994), Korea Republic (SukYoung *et al.*, 1995), Poland (Tylkowska and Dorna, 2001). *B. aclada* can also infect garlic (*Allium sativum* L.), leek (*A. porrum* L.), shallot (*A. cepa* var. *ascalonicum* Backer) and potato or multiplier onions (*A. cepa* var. *aggregatum* L.).

2.2.1. Description
Mycelium is septate, branched and hyaline when young. Sclerotia are frequently formed on natural substrata, but they are less in culture. The mature sclerotium has a narrow rind of round cells and not thick-walled empty cells and a large medulla of filamentous hyphae loosely arranged in gelatinous matrix. When germinated, it produces abundant conidiophores with conidia. Conidiopores emerges from the ruptured rind originated in the medulla (Sadeh *et al*., 1985). Sclerotia often form on the shoulders of affected bulbs and may be up to 10 mm in length. Sometimes they occur as solid crusts around the neck area (Lacy and Lorbeer, 1995). Conidia and conidiophores take on a smoky gray appearance in mass. Conidia are narrowly ellipsoidal and hyaline. They are borne on brown, rather short (about 1mm) conidiophores with side branches at the tips, each of which has many appullae that swell gradually at the tips to form conidia on fine denticles (Lacy and Lorbeer, 1995). Although it has been postulated that *B. aclada* and *B. byssoidea* a conspecific (Lacy and Lorbeer, 1995), though *B. aclada* is significantly different from *B. byssoidea* in some characters (Nielsen *et al.,* 2001b).

2.2.2. Symptoms and epidemiology
The disease generally appears after the bulbs are stored. The fungus grows down through the inner scales and partially causes decay of the bulbs before external injury appears. Infected tissues usually appear soft and watery at first, but later turn brown and become spongy and light in weight (Tabira *et al.*, 1999).

Furthermore, the disease reduces seed yield, thousand grain weight and seed quality (germination, conductivity and field emergence). Quality of seeds produced by the infected plants is significantly reduced (Rudolph, 1990b,c.; Tylkowska and Dorna, 2001).

A major source of the pathogen is the samples of infected seeds (Maude and Presly, 1977a; Bochow, 1981; Tylkowska and Dorna, 2001). The pathogen *B. aclada* remains internally in the seeds from infected plants and is viable upto 3½

years in storage at 10 ⁰C and 50% RH (Maude and Presly, 1977 a; Bochow, 1981). Seeds from 5% non-healthy onion bulbs may be infected with the pathogen under favorable meteorological conditions (Tylkowska and Dorna, 2001).

The fungus can be transmitted from infected seeds to seedlings and bulbs. Maude and Presly (1977 a), discussed the transmission of the pathogen in onions grown in U.K. Seedlings raised from diseased seeds become infected by mycelial invasion of the cotyledonary leaf tips from seed coat. The fungus, which remains attached to the cotyledons, can attack the living tissues of the leaves of seedling emerging from the soil (Fig.3). No symptom of the disease is observed on these leaves, fungus produces conidiophores and conidia only after the leaf tissue senesces and becomes necrotic. It invades the leaves of the plants successively; first it infects the leaves at the tip parts then grows downwards in the tissue and invades the neck of the onion bulbs, at harvest it penetrates deep in the neck tissues of maturing bulbs. The disease is mainly spread by conidia formed abundantly on conidiophores on plants in the fields under high humidity. However all seed infection may not result in seedling or bulb infection. Stewart and Franicevic (1994) obtained the same result in New Zealand. It is possible to detect latent infection of *B. allii* in onion bulbs, seed and sets using PCR-based method (Nielsen *et al*., 2001 a).

The disease does not spread from infected bulbs to healthy ones during storage but affected bulbs rot. The amount of neck rot in store is directly related to the percentage infection of onion seeds (Maude and Presly, 1977 b; Stewart and Franicevic, 1994).

Maximum seed infection occurs in the stage of full bloom (Vannacci and Gambogi, 1982). Cool and rainy weather conditions are important for the infection of flowering shoots (Rudolph, 1990 a). *Botrytis aclada* is pathogenic on onion
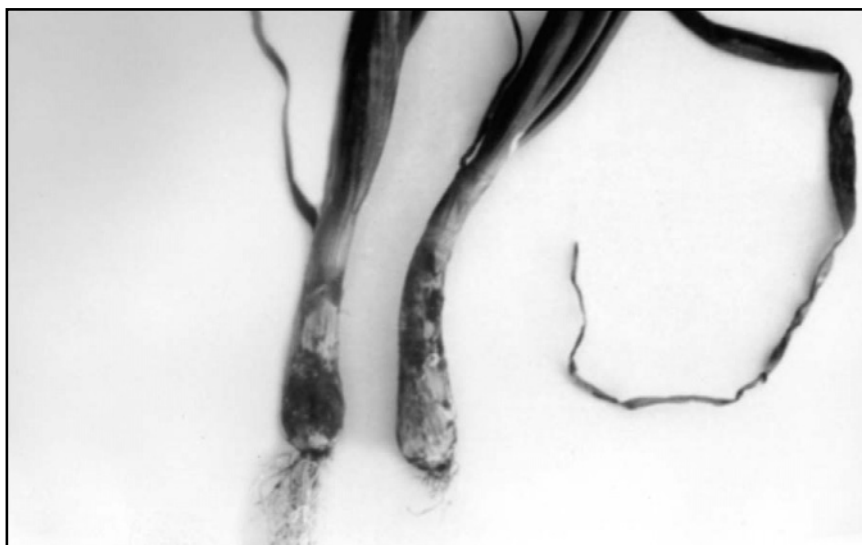
Fig. 3: Onion plants infected at the soil line by *Botrytis* species.

umbels and causes flower blight. Unopen umbels are less susceptible than open umbels to blighting. In humid conditions *B. aclada* forms lesions on onion seed stalks and expands to girdle the stalks and abort the umbels (Ramsey and Lorbeer, 1986 a,b). The pathogen needs free moisture in order to colonize uninfected tissues and long periods of continuous free moisture (24 h or more) at 21^{0}C induce a substantial amount of blighting of florets and immature seed capsules (Ramsey and Lorbeer, 1986 c).

Dry infected debris may remain on the soil surface after the crop has been cleared and in some cases rotted bulbs in the field may release sclerotia into the soil (Fig.4). However, the fungus does not survive in the soil on debris or as sclerotia for more than 2 years (Maude *et al.*, 1982).
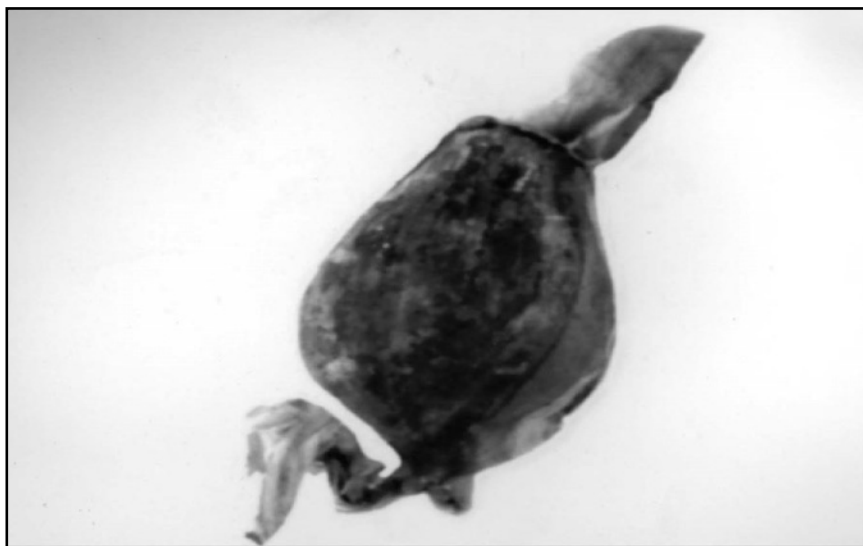
Fig. 4 : Infection on onion bulb at the soil line by *Botrytis* spp.

The disease is prevalent in the areas with cool, moist weather conditions before and during the harvest. It develops most rapidly between 15-20 °C. Fungal growth slows greatly at temperatures below 3 °C, but neck rot can continue to develop even at 0 °C over several months of storage.

2.2.3. Host Parasite Interaction

The ability of *B. aclada* to secrete *in vitro* and *in vivo* polysacharide-degrading enzymes involved in cell-wall breakdown is well known (Hancock *et al.* 1964; Magro *et al*. 1979; Mankarios and Friend, 1980; Kritzman *et al*. 1981; El-Zawahry *et al*. 1997). It has been suggested (Magro *et al*. 1983) that PG increased less rapidly, reached lower levels and with fewer isoenzymes in the white cultivars of onion which were less susceptible, than the red cultivar (Magro *et al*., 1983).

Onions are known to produce toxic substances in the scales, which give resistance to *B. aclada (*Walker *et al.* 1950). Toxic volatile substances are present

in the fleshy scales of all varieties tested. Phenolic substances present in the outer dry scales of colored bulbs are also antifungal. Catechol and protocatechuic acid provides resistance to colored onions against infection with *B. aclada* (Walker and Lindegren, 1924; Link and Walker, 1933).

As known, the oxidation products of phenols may be fungitoxic and their reactive compounds may inhibit the pectic enzymes produced by pathogen (Brydge *et al.* 1960; Hunter, 1974; Friè, 1976; Friend, 1977; Vance *et al.,* 1980). The resistance of onion cultivars to *B. aclada* is associated with the accumulation of the phenolic compounds and especially with the activation of peroxidase, which can oxidize phenols. Magro *et al.* (1983) reported that cell wall modifications (including the formation of lignin precursors and lignification) occurred more rapidly, retarding the progress of the *B. aclada* hyphae in the white onion cultivars possessing this mechanism. In the following year, Stewart and Mansfield (1984) suggested that onion bulbs resistance to colonization by *B. aclada* was due to poor germination, failure to produce distinct infection hyphae which is associated with accumulation of deposit of granular reaction material (RM) in underlying live cells. RM granules are osmiophilic aggregates formed between the plasma membrane and epidermal cell wall. McLusky *et al.* (1999) reported that the formation of RM was associated with early increases in peroxidase activity at reaction sites and striking polarisation of actin microfilaments and that feruloyltramine derivatives, which are the major autofluorescent components of RM, suppressed of flavonoid and anthocyanin accumulation in a zone of cells around those accumulating RM. However no antifungal activity was detected in identified feruloyl-3'-methoxytyramine (FMT), feruloyltyramine (FT).

Dimitriev *et al.*, (1990), were the first to determine a phytoalexin named as tsibulin 1d (1,3-dion 5-octyl- cyclopentane) in onion bulb scales in response to inoculation with *B. aclada*, but it did not accumulate at a high amount.

2.2.4.　Control

The removal of diseased onion crops from field in autumn, before numerous of sclerotia have developed, eliminates the source of pathogen (Maude *et al.*, 1982). High level of nitrogen increases infection of onion by the pathogen (Maude, 1980). It was recommended to apply one single dose of 75-90 kg N/ha early in spring (Rudolph, 1986). Practices that hasten curing include applying no nitrogen fertilizers later than about 8-9 weeks after seeding and achieving the proper plant density (about 550,000 plants per hectare or about 26 plants per linear meter of row on 46 cm row spacing) (Lacy and Lorbeer, 1995). Crop rotation of four years with a non-susceptible host reduced the risk of infection by *B. aclada* (Maude *et al.*, 1982; Lacy and Lorbeer, 1995). Shriveled seeds should not be used and only seeds that are free of *B. aclada* should be planted. Resistant cultivars were

determined by using transplants and bulbs (Vik and Aasveit, 1984; Miyaura *et al*., 1985; Ahmed *et al*., 1992; El-Zawahry *et al*., 1997). There is no report about resistance to seed infection by *B. aclada.*

Under *in vitro* conditions, fungal antagonists like *Gliocladium roseum*, *Trichoderma hamatum*, *T. harzianum*, *T. koningii* and a biological product, Promat (*Trichoderma* based) are effective against *B. aclada* (Rod, 1984; Köhl *et al.,* 1991; Özer *et al*., 1995; El-Neshawy *et al*., 1999). Neck rot is controlled in the bulbs resulting from dip treatment of onion seedlings in Promat suspension before planting and three spray applications at 15 day intervals four months after planting (El-Neshawy *et al*., 1999). A different fungal antagonist, *Gliocladium atrum* was found to be effective for reducing *B. aclada* infection under pot conditions (Nielsen *et al*., 2001 c).

Several fungicides have been tested for inhibition of *B. aclada* in *in vitro* conditions. Benomyl (Rod, 1980; El-Shehaby et al., 1987), iprodione, carbendazim, carbendazim+thiram (Rod, 1980), procymidone and vinclozolin (Rod, 1980; Özer *et al.*, 1995) have proven effective for control of mycelial growth. Benomyl (Tahvonen, 1983; El-Shehaby *et al*., 1987; Özer and Ömeroðlu, 1995), iprodione (Kritzman, 1983), thiophonate methyl (Barnoczkine-Stoilova, 1984; Rod and Janyska, 1984), vinclozolin (Kritzman, 1983; Rod and Janyska, 1984; Özer and Ömeroðlu, 1995) controlled the disease when they were sprayed to onion sets, transplants and nurseries. However there is a little commercial prospect for elimination of *B. aclada* from seeds because of its survival in seeds for a long period as 3.5 years (Maude and Presly, 1977 a; Maude, 1980). It is reported that seed treatment with Benomyl is effective to *B. aclada* infection. Benomyl (2 kg/kg seed) considerably reduced the *B. aclada* infections on onion seedling, but was not successfull in eliminating it completely (Bochow, 1981). In addition, treatment with thiram (3 kg/kg seed) proved less efficient when seed inherently contaminated with *B. aclada* had been used exclusively. Good results were obtained when using a compound preparation containing carbendazim and thiram (Bochow, 1981). Some other fungicides and other seed treatments should also be tested for the control of this pathogen on onion seed.

2.3. *Fusarium oxysporum* Schlecht. f. sp. *cepae* (Hans) Snyder & Hans.

Fusarium oxysporum f. sp. *cepae* causes the basal plate rot in onion. The pathogen exists in nearly every onion-growing area of the world including Italy, Japan, South Africa, Turkey and the United States (Havey, 1995; Köycü and Özer, 1997). Incidence of *Fusarium* basal rot generally occurs during the development of seedlings and bulbs, and in storage and ranges from 2.9 to 80 % depending upon the time of year, environmental conditions, cultivars and level of inoculum (Lacy

and Roberts, 1982; Somkuwar *et al.,* 1996; Stadnik and Dhingra, 1996). Other cultivated *Allium* species such as shallots (*A. cepa* L. var *ascalonicum* Backer), Welsh onion (*A. fistulosum* L.) and chives (*A. schoenoprasum* L.) also may suffer losses (Havey, 1995).

2.3.1. Description

The pathogen (*F. oxysporum* f. sp. *cepae*) produces chlamydospores, macroconidia and microconidia. Chlamydospores are round thick-walled, and are formed abundantly in soil. Macroconidia are short to medium in length, falcate, thin-walled, slightly tapered at the end, and usually 3-4 septate. Microcondia are usually non-septate, oval to reniform in shape, and abundant in culture. *F. oxysporum* f. sp. *cepae* isolates from different onion seeds, fields of different regions or countries show different degrees of virulence (Villevieille, 1996; Özer and Köycü, 1997). However separate races have not been identified (Havey, 1995).

2.3.2. Symptoms and Epidemiology

The visual symptoms of the disease can be observed on leaves, roots, sets, basal stem plate, bulb scales, mature bulbs and dormant bulbs in store (Lorbeer and Stone, 1965; Abawi and Lorbeer, 1971b, c; Tahvonen, 1981; Köycü and Özer, 1997). *F. oxysporum* f. sp. *cepae* reduces seed yield, germinability, 1000 seed weight (Barnoczki-Stoilova, 1986) and generally kills young seedling at pre- and post-emergence stages (Naik and Burden, 1981; Kodama, 1983; Srivastava and Qadri, 1984; Özer and Köycü, 1997). Symptoms on set bulbs are difficult to observe. On set bulbs, above ground symptom of the pathogen is chlorosis of the leaves. This chlorosis leads to tip necrosis and eventually progresses to entire leaf necrosis and plant deaths including the mature bulbs (Havey, 1995; Özer and Ömeroðlu, 1995; Brayford, 1996). However any symptoms do not occur on leaves until the sets are completely developed (Köycü and Özer, 1997). If the sets containing the pathogen are used for the bulb production, it is possible to see leaf chorosis or basal rot symptoms on mature bulbs. The infection within basal plate causes root death and root abscission. The pathogen causes a brown discoloration of the basal plate tissue. In severe cases, it infects the basal portions of the bulb scales and white mycelium can be observed on the basal portions of the exterior bulb scales (Cramer, 2000). The later symptom can also appear on the stored bulbs (Fig.5).

The pathogen can also cause latent infection on mature bulbs and the plants may confine the pathogen to the stem plate. In such cases, the fungus reduces the weight of the bulbs at harvest (Abawi and Lorbeer, 1972). Furthermore an early infection that does not result in conspicuous symptoms may weaken the plant and predispose it to other stresses or diseases (Fantino and Shiavi, 1987; Stadnik and Dhingra, 1995, 1997).

F. oxysporum f. sp. *cepae* is seed-borne in onion (Kodama, 1983; Abd-ElRazýk *et al.,* 1990; Boff *et al.,* 1995; Köycü and Özer, 1997; El-Zawahry *et al.*, 2000). However it is not always isolated when the onion seeds are screened for the fungi. But these seeds are not considered as pathogen-free. Köycü and Özer
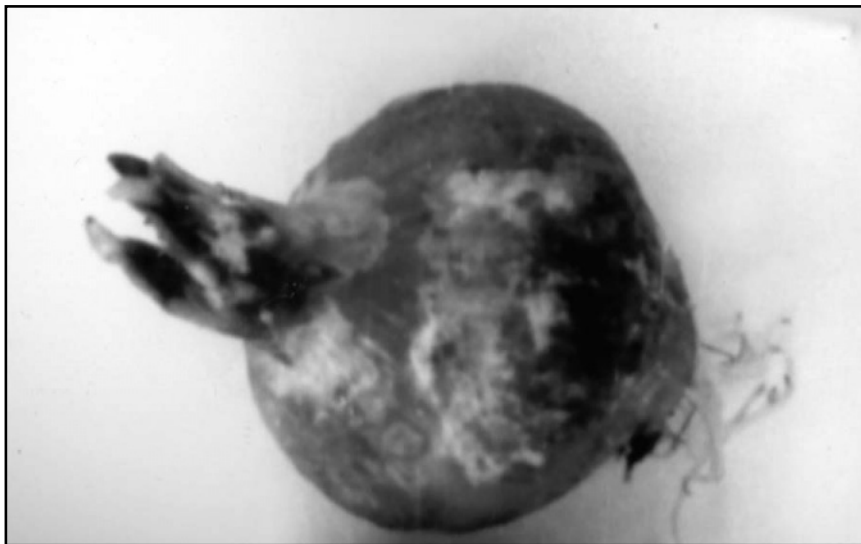
Fig. 5 : Advanced infection by *Fusarium oxysporum* f. sp. *cepae* of onion bulb.

(1997) isolated *F. oxysporum* at very low incidence in the embryo tissue of onion seeds, only from the samples of one region of Turkey; seed samples from six other regions, having different climatic conditions, did not contain this fungus; whereas, the roots and bulbs of onion sets, developed from all seed samples grown in sterile soil, were infested with this pathogen. This fungus could be transmitted from the seeds to onion sets. Other pathogens like *Aspergillus niger* in seeds, which grows very rapidly, probably inhibited the development of *F. oxysporum* in artificial media; this pathogen also was a natural component of the soil mycoflora. In conclusion, *F. oxysporum* is transferred from seed to soil and it perenates in the soil for a longer or shorter period and then to host as local or systemic infection such as is also the case with other soil inhabitants and soil invaders. However, it is already known that *Fusarium* spp. have a poor ability to compete with other microflora in natural soil (Steiner and Lockwood, 1969; Ford *et al.,* 1970). Biotic and physical factors in additions to the size of the population of *F. oxysporum* f. sp. *cepae* determine the basal rot potential of naturally infested organic soil under field conditions (Abawi and Lorbeer, 1972). The pathogen can also be spread by infected debris, infected soil (Abawi and Lorbeer, 1971a), irrigation water, farm equipment (Everts *et al.,* 1985) and onion transplants (Kodama, 1983).

Mechanical wounding, resulting from cultivation from hand weeding (when weed and onion roots are growing together) and from clipping plant roots before transplanting. However the pathogen can cause the disease on unwounded bulbs also. In Colorado, *Fusarium* basal rot is often associated with maggot infestation, especially seed-corn maggots (*Delia platura* Mergen). However they generally appear to be secondary invaders of diseased bulbs in onion fields (Everts *et al.*, 1985).

The optimum soil temperature for the development is between 28 and 32°C; but the disease can occur at a soil temperature range of 15-32°C (Abawi and Lorbeer, 1972; Kodama, 1983). The optimum pH for growth is 6.6, but growth can occur at a pH range of 2.2 to 8.4 (Walker and Tims, 1924).

2.3.3.   Host Parasite Interactions

F. oxysporum f. sp. *cepae* releases pectic enzymes including exo- PG and endo pectin-transeliminase (endo-PTE) that work to break down pectin in cell wall of the onion during the infection. Exo-PG activity is quite important for the initial infection of the bulb (Holz and Knox-Davies, 1985). The apoplast sugars released from the bulb tissue feeds the fungus and ensures its growth and reproduction and thus the decay becomes evident (Holz and Knox-Davies, 1986). The pathogen has the ability to produce pectic enzymes during the seed colonization. PG is an important determinant during onion germlings infection by this fungus. Because the pH of infected tissues (pH 5) is close to PG optimum. In addition the ability of *F. oxysporum* f. sp. *cepae* to produce multiple PG and Pectin Lyase (PNL) isoenzymes on seeds may be considered as advantage in its versatility and expression of virulence during different stages of onion growth (*Unpublished data*).

2.3.4.   Control

The disease can be controlled through crop rotation, host plant resistance, biological control and fungicide applications.

Crop rotation with a crop like maize or spring wheat reduces soil inoculum levels and onion bulb rot. A crop rotation of four year with a non-susceptible host has been recommended (Havey, 1995). Numerous onion lines and cultivars were tested for the resistance to *Fusarium oxysporum* f. sp. *cepae*. These lines and cultivars and their reactions under different experimental conditions to the disease are listed in Table 1.

Disease management strategies should be based on the relationship between onion maggots, seed-corn maggots, and *Fusarium* basal rot and emphasize the importance of minimizing stress and injury the bulbs (Everts *et al*., 1985).

Under *in vitro* conditions fungal antagonists *Beauveria bassiana* (YoungGoo *et al.,* 1996), *Trichoderma harzianum* (Abouzaid *et al.,* 1993; Rajendran and Ranganathan, 1996), *T. hamatum*, *T. koningii*, *T. pseudokoningii* and *T. viride*, and bacterial antagonists, *Pseudomonas fluorescens* and *Bacillus subtilis* (Rajendran and Ranganathan, 1996) inhibited mycelial growth of the pathogen. A combination of *T. viride* and *P. fluorescens* were most effective for reducing *Fusarium* basal rot incidence under pot and field conditions (Rajendran and Ranganathan, 1996).

Different fungicides have been tested for eradication of *F. oxysporum* f. sp. *cepae* on onion seed. Benomyl (Gupta *et al*., 1984; Barnoczkine-Stoilova, 1988; Abd-ElRazik *et al*., 1990; Özer and Köycü, 1998), carbendazim, carboxin hydroxyquinoline, iprodione and metoxymethyl mercury chloride (Barnoczkine-Stoilova, 1988; Abd-ElRazik *et al*., 1990), thiram, benomyl+thiram, prochloraz

TABLE 1 :
The reactions of some cultivars or lines to *Fusarium* basal rot
disease at different experimental conditions and cited references

Cultivars or Lines	Experimental conditions	Reactions	References
'XPH 419', XPH 70',	Field	Resistant	Lacy and Roberts 1982
'W404'	*In vivo*	Resistant	Krueger *et al.* 1989
"Baia Oura A659", "Norte 14"	*In vitro*	Moderate resistant	Stadnik and Dhingra, 1994
'Hybrid 1', ,IIHR Yellow', 'Sel. 29'	*In vitro* and field	Resistant	Samkuwar *et al.* 1996
"Bola Precoce", "Roxa do Barreiro", "Cebola de Varao", "Crioula", "Monte Alegre", "Pera IPA 3", "Roxa IPA 3", "Texas Grano 502"	Harvest	Resistant	Stadnik and Dhingra, 1996
'Cebola de Varao'	Storage	Resistant	Stadnik and Dhingra, 1996
SR 2308-2	Field	Resistant	Thornton and Mohan, 1996
'W207C', 'W434A', 'W434B', 'W435A', 'W435B', 'W440A', 'W440B', 'W446A', 'W446B', 'W447A', 'W447B', 'W460A', 'W460B'	Field	Very high resistant	Goldman 1996
'Akgün 12', 'Alex'	*In vitro*	Moderate resistant	Özer, 1998
'IIHR-141', ,IIHR-506', 'Sel 13-1-1'	*In vitro* and field	Resistant	Ganeshan *et al.* 1998
"Dawn", "Impala", "La Nina", "Navigator", "NuMex Casper", "NuMex Centric", "Riviera", "Utopia"	Field	High resistant	Cramer 2000
'NuMex Dulce', 'NuMex Vado', 'Aspen', 'Frosty'	Field	Moderate resistant	Cramer 2000

and tebuconazole (Özer and Köycü, 1998) increased seed germination and inhibited mycelial growth of *F. oxysporum* f. sp. *cepae in vitro* conditions. Wicks and Philp (1985) suggested that iprodione and vinclozolin reduced seed germination of the

onion cultivars, White Spanish and Goldberg and caused the seedling to stunt. Seed treatment with thiram (2g/kg seed) combined with thiram soil treatment at 5 g/m² followed by another direnching (0.2% thiram) 30 days after sowing, gave the best control of the pathogen (Gupta *et al.*, 1987). In greenhouse trials, Roberti *et al.* (1989) found that maneb resulted in development of the highest percentage of healthy plants, alone and in mixture with thiophonate-methyl it gives the highest percentage of germination in artificially infested soil with *F. oxysporum* f. sp. *cepae*. On the other hand, it was reported that carbendazim and benomyl protected seedlings from infection by this pathogen (Abd-ElRazik *et al.*, 1990). In the following years, Benomyl+thiram (1.50+4.05 g ai/kg seed) and prochloraz (1.35 cc ai/kg seed) were found to control infection by *F. oxysporum* on seeds and in soil. Otherwise, organic amendments of naturally infested soil by this pathogen with the stalks of sunflowers, alfalfa and Hungarian vetch reduced the disease incidence on the sets developed from seeds. In addition sunflower stalks increased heterotrophic fungal population in the soil (Özer *et al.*, 2002). Further research should be continued in the field.

3.   Other possible seed-borne pathogens

Other fungi isolated from onion seeds are listed in Table 2. Some of these fungi, like, *Alternaria alternata*, *Aspergillus alutaceaus*, *A. flavus*, *Beauveria bassiana*, *Cladosporium cladosporioides*, *Colletotrichum dematium*, *Curvularia lunata*, *Drechslera australiensis*, *Humicola fuscoatra*, *Stemphylium botryosum*, *Trichoderma hamatum*, *T. pseudokoningii* and *Trichothecium roseum* *etc*. are not considered as pathogen of any known onion disease. Some others, such as *Alternaria porri, Botrytis byssoidea*, *B. cinerea*, *B. squamosa* and *Stemphylium vesicarium* sometime cause formation of several lesions on the leaves, and also reduce seed yield.

Lesions may develop on the leaves by *A. porri* and *S. vesicarium* and may even develop on seed stalks and floral parts of seed onion. Seeds may not develop or are shriveled (Nolla, 1927; Munoz de Con, 1985; Castellanos Linares, 1986; Aveling *et al.*, 1993; Jakhar *et al.*, 1996; Lakra, 1999). It is yet not known as to whether *A. porri* is transmitted from infected seeds to seedling, sets and bulbs (Aveling, 1998). However, it has been reported that the survival of *S. vesicarium* was only 28 % in seeds under laboratory conditions and survival under field conditions is nil and that it could not be re-isolated from the seeds (Jakhar *et al.*, 1996).

Table 2.

Seed-borne fungi in onion and cited references

Fungi species	References
Alternaria alternata (Fr.) Keissler	Miura, 1985; Rod, 1983; Boff *et al*.,1995
Alternaria porri (Ell.) Cif	Miura, 1985; Aveling *et al*., 1993; Boff *et al*., 1995; Tylkowska and Dorna, 2001.
Aspergillus alutaceaus Berk. and Curt	Köycü and Özer, 1997
Aspergillus flavus Link ex Gray	Gupta *et al*., 1984
Aspergillus fumigatus Fres	Hayden and Maude, 1994a
Beauveria bassiana (Balls) Vuill	Köycü and Özer, 1997
Botrytis bysoidea Walker	Stewart and Franicevic 1994
Botrytis cinerea Pers.: Fr.	Rod, 1983; Stewart and Franicevic 1994; Rudolph and Bräutigam, 1990; Boff *et al*., 1995; Tylkowska and Dorna, 2001
Botrytis squamosa Walker	Ellerbrock and Lorbeer, 1977 a; Stewart and Franicevic, 1994; Boff *et al*., 1995
Clodosporium allium-cepae (Ronojevic) Ellis	Jordan *et al*., 1990; Boff *et al*., 1995
Clodosporium cladosporioides (Fres.) de Vries	Köycü and Özer, 1997
Colletotrichum circinans (Berk.) Vogl	Rod, 1983
Colletotrichum gleosporioides (Penz.) Penz and Sacc.	Boff *et al*., 1995
Colletotrichum dematium (Pers. ex Fr.) Grove	Boff *et al*., 1995
Curvularia lunata *(Wakker) Boedijn*	Boff *et al*., 1995
Fusarium avenaceum (Corda ex Fr.) Sacc.	Rod, 1983; Tylkowska and Dorna, 2001
Fusarium equiseti (Corda) Sacc	Rod, 1983
Fusarium moniliforme Sheldon	Abd-ElRazýk *et al*., 1990
Fusarium moniliforme var. subglutinans Wr and Reink	Vannacci, 1981
Fusarium solani (Mart) Sacc.	Rod, 1983; Boff *et al*., 1995
Humicola fuscoatra Traaen	Köycü and Özer, 1997
Penicillium cyclopium Wastling	Rod, 1983
Rhizoctonia solani Kühn	Abd- ElRazýk *et al*., 1990; Boff *et al*., 1995
Rhizopus nigricans Her.	Rod, 1983
Rhizopus stolonifer	Boff *et al*. ; 1995
Stemphylium botryosum Wallr	Rod, 1983; Köycü and Özer, 1997; Boff *et al*.; 1995 Tylkowska and Dorna, 2001
Stemphylium vesicarium (Wallr.) Simmons	Aveling *et al*., 1993; Jakhar *et al*. 1996
Trichoderma harzianum Rifai	Köycü and Özer, 1997
Trichoderma pseudokoningii Rifai	Köycü and Özer, 1997
Trichothecium roseum (Pers) Link ex Gray	Rod, 1983

B. byssoidea causes mycelial neck rot (Walker, 1925) but it does not show any infection of the umbels (Ramsey and Lorbeer, 1977 a). However it can cause severe infection in seedling leaves (Presly, 1985).

B. cinerea and *B. squamosa* causes blight of all floral structures and immature seed capsules. As a result, seeds shrivel and die. Wet

weather during the periods from anthesis to seed harvest appears to favor disease development (Crowe *et al.*, 1995). Lesions caused by *B. squamosa* on onion seed stalks are very important, expand sufficiently to girdle the stalk and abort the umbel (Ellerbrock and Lorbeer, 1977a; Ramsey and Lorbeer, 1977 a,b,c). However *B. squamosa* is unable to demonstrate disease transmission to seedling grown from infected seeds (Ellerbrock and Lorbeer, 1977b). Furthermore, *B. squamosa* seed infection was not detected in onion seed in Germany (Rudolph and Bräutigam, 1990).

Cladosporium allii-cepae can occur on onion seed at very low level and possibly be a source of initial inoculum. It occurs on the surface of the testa and is therefore unlikely to survive for long periods (Jordan *et al.,* 1990). It has been demonstrated that the incidence of contaminated seeds was low despite severe symptoms in the flowering inflorescens of onion dusted with conidia of *C. allii-cepae* (Jordan *et al.*, 1990). In addition no infected onion seeds were obtained from naturally infected plants in the field.

Fusarium spp. and *Rhizoctonia solani* known seed-borne fungi cause damping-off where onions are grown as a continuous crop in seedbeds, field and or garden (Sumner, 1995 a,b). Among these, *F. moniliforme* var. *subglutinans* was re-isolated from the seedlings developed from infected seeds by this pathogen (Vannacci, 1981). *F. moniliforme* and *R. solani* are pathogenic and caused pre- and post-emergence damping-off of seedlings (Abd-ElRazik *et al.*, 1990). *Penicillium cyclopium* may appear on the wounded or unwounded bulbs in the field and storage (Sumner, 1995d). *A. fumigatus* was found on the leaves of the onion grown from *A. niger*-inoculated or non-inoculated seeds and it was also abundant in the air mycoflora of Sudan (Hayden *et al.*, 1994b). It was suggested that *A. niger* and *A. fumigatus* competed for the same niche in the onion leaf mycoflora, but *A. niger* was the more competitive (Hayden *et al.*, 1994b). *Colletotrichum circinans* causes the smudge on the bulbs of white onion cultivars. *C. gleosporioides* is known as the causal agents of twister or anthracnose disease (Hill, 1995; Boff, 1996). *Rhizopus nigricans* and *R. stolonifer* cause mushy rot on the bulbs, particularly in the neck regions and require wounds to invade the onion bulbs (Sumner, 1995e; Abdel-Sater and Eraky, 2001). Although all these

fungi were isolated from the surface of onion seeds, it is not known whether they are important in the disease cycle and transmission.

Seed-borne infection is relevant only if infected seeds germinate and transmit the pathogen to plant which then act as primary disease source in crops (Maude, 1980; Neergaard, 1979). Further studies needs to be done to determine the extent of seed source of these pathogens.

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9

Management of Sugarbeet Diseases

S.N. Srivastava

ABSTRACT: The diseases of the sugarbeet (*Beta vulgaris* L) are one of the major constraints in the profitable commercial cultivation of crops in the country. Under Indian conditions, about 10-15% of the crop is destroyed by the diseases. Out of 25 diseases known in the country, about 15 are economically important. Among the diseases caused by fungi, seed/seedling disorders, foliar diseases, root rots of adult plants and nematodes both in root and seed crop are more destructive. Fortunately, the extent of damage caused by bacteria and viruses is negligible. Seedling afflictions also known as damping-off, black leg, seedling blight, seedling root rot, collar rot are of great importance. Both pre- and post-emergence mortality destroying 15-30% seeds/seedlings are observed. The pathogens envolved are *Phoma betae*, *Pythium* spp., *Sclerotium rolfsii*, *Rhizoctonia solani*, *R. bataticola*, *Fusarium* spp. and *Alternaria* spp. Seed/Seedling diseases can be effectively managed by conventional seed treatment with fungicides, seed polishing and seed pelleting with mixture of two fungicides or incroportion of bio-agents. Among foliar diseases, Cercospora leaf spot (*Cercospora beticola*) is a major disease of root crops in plains reducing both root yield and sugar production by 33% and 44%, respectively. Alternaria leaf spot (*Alternaria alternata, A. brassicae*) also damages up to 30% leaf area. Powdery mildew (*Erysiphe betae*) is a disease of warm and dry weather and reduces the root yield by 20-25%. These can be managed by spraying of fungicides like Benlate, Bavistin, Thiabendazol, wetable sulphur, Dithane M-45 and Dithane Z-78. Adult sugarbeet roots are affected by a number of soil borne diseases. Sclerotial root rot (*Sclerotium rolfsii*) is the most destructive disease causing about 50% damage of roots. Other diseases are dry root rot (*Rhizoctonia solani*) and charcoal root rot (*R. bataticola*) causing 15-30% damage of roots. Affected roots from these diseases are unfit for sugar extraction. These diseases can be managed by soil drenching of fungicides like PCNB, Bavistin Vitavax and Thiram and by manipulation of cultural practices. Biological agents like *Gliocladium virens*, *Trichoderma harzianum* and *T. viride* have been found effective in reducing root rots.

1. Introduction

From the earliest time of history, sugarbeet has formed a basic food for man, animal and plant. It is an important part of the human diet, providing energy to maintain body temperature activity. Additioally, it is also widely used as a sweetener and preservative for other foods like beverages, confectionaries, canned foods and pharmaceuticals. World-wide, sugar is commercially manufactured chiefly from two sugar crops, sugarcane and sugarbeet. Globally, sugarbeet crops contribute about 36% of the total centrifugal sugar produced, with the remaining 64% from sugarcane.

Sugarbeet (*Beta vulgaris* L.), a specialized type of beet belonging to family Chenopodiaceae, is a biennial plant. Essentially, it is a crop for temperate climates, but now it can be grown successfully in a wide range of climates on different soils between latitudes of 30º and 60º N, as winter and winter/summer crops. In India, research has shown that the successful cultivation of the crop is feasible as a supplementary sugar crop, particularly in northwestern India. During 1972-1998, the crop was commercially grown in the Sriganganagar area of Rajasthan and was processed for sugar production during April and May every year with a good recovery of sugar. Besides sugar, it also provides valuable by-products, *i.e.* beet tops and molasses being used as cattle feed and in the fermentation industry for the production of vitamin B-complex and other pharmaceutical products.

Globally, diseases are one of the major constraints in the profitable yield of sugarbeet in the form of tonnage and sugar content which can be economically be processed into commercial sugar. About 16-20% of the crop is destroyed by diseases every year. The diseases of the sugarbeet have played an extremely important role in the current distribution of the beet sugar industry and sugarbeet crops in most of the sugarbeet growing countries (Duffus and Ruppel, 1993). The crop is subject to attack by these diseases from the time of seed-sowing, until the harvest of the crop. All parts of the sugarbeet plant (seeds, seedlings, roots and foliage) are susceptible to attack by a number of diseases which reduce the quantity and quality of roots and seed. World-wide over 50 diseases are known to affect sugarbeets, of which nearly 20 are of economic importance (Mukhopadhyay, 1987). With

the expansion in the area under sugarbeet production world-wide, the diseases have increased in number and severity.

In India, initially two main hurdles, *viz.*, poor seed production in the country and diseases of crop arose to limit beet culture. Now with the success of seed production in Kashmir (Jammu & Kashmir), Kumaon hills (Uttaranchal) and Kalpa hills (Himachal Pradesh), it is no longer a major problem. This leaves only the diseases of sugarbeet to be dealt with. The introduction of a temperate crop in a tropical and sub-tropical climate poses many important pathological problems due to prevailing high temperature. The conditions suitable for growth and development of the crop and the succulent nature of its foliage and roots are also favourable for quick development, proliferation and spread of the diseases. The major deterrents in culturing are diseases caused by fungi, of which seedling afflictions and root rot in the plains, leaf spots and nematode disorders both in the plains and the hills are most destructive. Fortunately, the extent of the damage caused by bacteria and viruses are negligible, while fungi and nematodes are proving limiting factors in the profitable cultivation of the crop in the country. However, the maladies like rhizomania and cyst nematodes which have not been recorded thus far, may pose serious problems in the future if the crop is taken up in larger areas on a commercial scale in the country, and if for sowing purposes, a lot of sugarbeet seed is imported from other countries. In India, about 10-15% of the crop is damaged by diseases. About 25 diseases are known to occur in the country out of which 15 are economically important.

The new introduction of the sugarbeet crop poses many serious disease problems in the country, and has attracted the attention of many plant pathologists. Lot of excellent research work on various aspects of sugarbeet pathology has been done and a number of research papers and review articles have been published (Agnihotri, 1990; Mukhopadhyay, 1974, 1987; Singh *et al.,* 1971, 1974, 1975; Srivastava, 1998, 2000).

The aim of this review is to provide a complete and upto date information available on the different diseases of sugarbeet and their management with particular reference to India. For the sake of convenience, the author has discussed the diseases of major and minor importance in the following sections.

2.   Pathogenic Diseases

Pathogenic diseases also known as infectious diseases, are caused by living agents. Such diseases are capable of being transmitted from infected to healthy plants under favourable conditions. Pathogenic diseases are caused by fungi, bacteria, viruses, phytoplasma and nematodes. Among these plant pathogens, fungi and nematodes are major deterrents in India while other pathogens are of great economic importance in other sugarbeet growing countries. Therefore, in the present review, only diseases caused by fungi and nematodes have been described.

3.   Seed Borne Diseases

2.1. Seed Mycoflora

Like other crops, sugarbeet seeds harbour a number of fungi which are known to cause considerable damage to seeds, sprouting seeds and seedlings (Kimmel, 1995; Prince, 1986; Srivastava, 1998, 2000; Tomic, 1994). In India, it includes many known seed borne pathogens like *Cercospora beticola*, *Phoma betae*, *Rhizopus oryzae*, *Alternaria alternata* and *Fusarium* spp. (Singh *et al*. 1973, Srivastava and Tripathi, 1998a, 1999). Among these pathogens, *P. betae* (teleomorph stage - *Pleospora bjoerlingii*) is the most important seed-borne pathogen causing damping-off, leaf spot, stem, crown and storage rots. The pathogens, *P. betae*, *C. beticola* and *Verticillium* sp., have been detected in many seed lots of sugarbeet imported from other countries. Presence of *P. betae* was detected in many imported Maribo varieties, Ramonskaya produced in Kashmir and many varieties grown in Mukteswar. During 1997-98, some abnormal and discoloured seeds from Mukteswar collected by the author have shown 72-100% of infection of *P. betae* (Fig. 1). Such seeds upon germination, have produced pre- and post-emergence mortality of seedlings (Srivastava and Tripathi, 1998a).

The elimination of surface-borne pathogens was attempted for the first time by polishing seeds using a small seed polisher designed and fabricated at IISR Lucknow. Polishing of seeds significantly
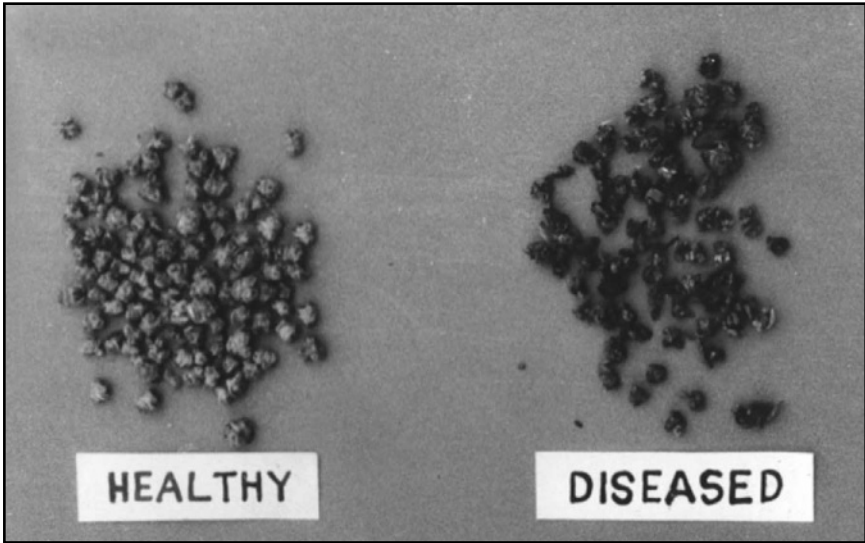
Fig. 1:   Healthy and diseased sugarbeet seeds.
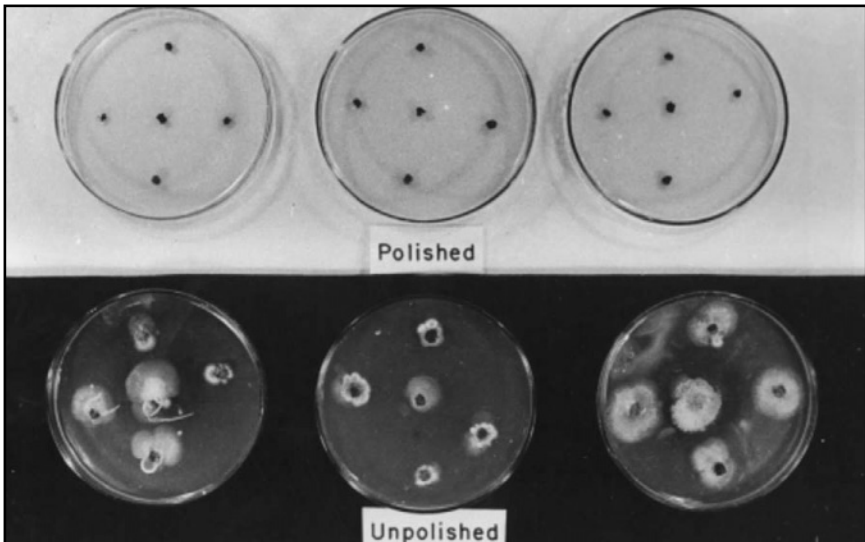
Fig. 2:   Effect of polishing on surface-borne fungal flora of sugarbeet seeds.

reduced, but did not eliminate both pathogenic and saprophytic mycoflora (Fig. 2). However, polishing of sugarbeet seed by rubbing to remove cortical tissues strikingly reduced the mortality of seedlings due to *P. betae* and *Fusarium* spp. in field (Singh *et al.*, 1973; Leach and Mac Donald, 1976).

Seed treatment of sugarbeet seeds with various fungicides has been found very effective for the elimination of seed mycoflora and better seedling stand in the field. In the country, of the various fungicides evaluated as seed treatment, organomercurials (Aretan, Ceresan), when used as steeping or slurry at 0.1 or 0.2% concentration, eliminated all the mycoflora of sugarbeet seeds. Species of *Alternaria, Fusarium* and *Rhizopus* are not eliminated by systemic fungicides like Benlate, Vitavax and Demosan; however, the population of the fungi could be reduced to some extent. Non-mercurial fungitoxicants like Dithane Z-78, PCNB, Captan and Thiram eliminated all fungi except *Alternaria* and *Fusarium* spp. (Singh *et al.,* 1973; Upadhyay *et al*., 1976; Srivastava and Tripathi, 1999). The seed treatments with a combination of fungicides provide better elimination of seed fungi than use of a single fungicide. Among various methods of seed treatment, incorporation of fungitoxicants in seed pellets most improved the efficacy of treatment (Sen *et al.,* 1974). In other beet growing countries, Thiram is commonly used for seed treatment (Payne, 1986; Dewar *et al.,* 1988; Hubbell and Paul, 1993; Eori, 1994).

3.2. Seedling afflictions

Seedling afflictions of sugarbeet known to its various names as black leg, damping-off, collar rot, seedling blight, seedling root rot *etc*. caused by different seed and soil borne pathogens have been reported world-wide wherever the crop is grown. Pathogens involved are *Aphanomyces cochlioides* (Lewis and Papavizas, 1971), *Phoma betae* (Srivastava and Pandey, 1979), *Pythium* spp. (Singh *et al.*, 1971; Sen *et al.,* 1974), *Rhizoctonia solani* (Singh *et al.,*1971), *R. bataticola*; Pycnidial stage-*Macrophomina phaseolina* (Sen *et al.*, 1974), *Fusarium* and *Alternaria* spp. (Mukhopadhyay and Sharma, 1968; Sen *et.al*., 1974), *Cylindrocladium betae* (Rama, 1981) and *Fusarium chlamydosporum* (Srivastava *et al.,* 1999). Of these, the first two are prevalent in temperate regions and others are ubiquitous in nature. Due to attack of these pathogens, some seedlings are killed before emerging from soil surface (pre-emergence phase) while other seedlings are dead after emergence (post-emergence phase). *Pythium* spp. and *P. betae* produce pre- and post-emergence damping-off while

others produce mostly post-emergence damping-off seedling diseases. Among the *Pythium* spp., *P. aphanidermatum* is mainly responsible for causing damping-off disease. However, other species *viz*., *P. ultimum*, *P. debaryanum*, *P. butleri*, *P. irregulare, P. oligandrum, P. splendens,* and *P. spinosum* have also been reported to cause the disease (Pandey and Agnihotri, 1983; Abada, 1994). Four species of *Fusarium*, *viz*., *F. oxysporum, F. moniliforme, F. avenaceum* and *F. chlamydosporum* have been found associated with sugarbeet seedling blight (Rama 1981; Srivastava *et al.*, 1999). Among *Alternaria* spp. *A. alternata* (syn. *A. tenuis*) *A. tenussima, A. dendritica, A.chartarum* and *A. harzii* are involved in causing seedling root rot but these are mild pathogens to germinating seedlings (Vesaley, 1977).

The extensive survey of seedling diseases, isolations from moribund seedlings and pathogenicity conducted with isolates of different fungi (Sen *et al.* 1974; Srivastava and Pandey, 1979; Rama, 1980; Srivastava and Tripathi, 1999) have shown that damping-off, seedling blight, collar rot and seedling root rot syndrome can be incited by *P. aphanidermatum*, *P. betae*, *R. solani*, *S. rolfsii*, *R. bataticola, Fusarium* spp. and *Alternaria* spp. The whole gamut of pathogens with low-temperature tolerance (*P. betae*) and with high temperature tolerance (*Pythium* spp., *R. solani*, *S. rolfsii*, *R. bataticola, Fusarium* spp. and *Alternaria* spp.) have been found to be responsible for poor sugarbeet stand in field. About 15-30% seedlings are destroyed by these pathogen in the field. Often more than one pathogen are isolated from a diseased seedling. Pathogens in association cause severe damage to seedlings than single pathogen. Some of these pathogens like species of *Fusarium, Alternaria* and *Cylindrocladium* are weak pathogens and diseased seedlings often recover from initial set back. The absence of *A. cochlioides* causing black leg in the country is due to an unfavourable climate. *P. betae*, *R.solani*, *R. bataticola* and *Fusarium* spp. are more preponderent in India as compared to other parts of the world.

The general symptoms on seedlings include initial shrivelling of primary roots a little below the collar-region. The number of root hairs are reduced and affected roots show brown to black apical necrosis which extends towards base (Fig. 3). Simultaneously, the young leaves develop acute chlorosis and the midvein and basal region
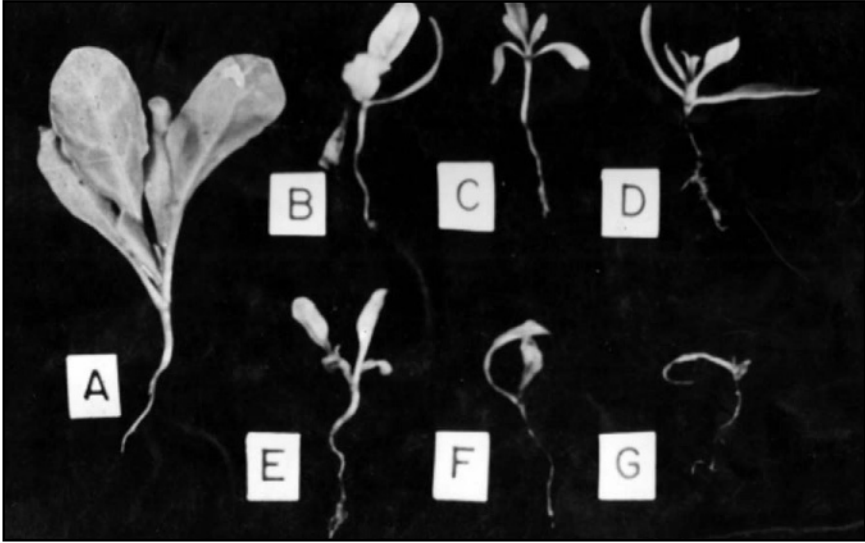
Fig. 3:   Seedling diseases of sugarbeet.

of leaf lamina remain green. Seedlings affected by *P. aphanidermatum* and *P. betae* produce both pre- and post-emergence mortality. In pre-emergence phase, germinated seedlings including plumule and radical are killed below the soil surface or even before the hypocotyl has broken the seed coat. The post-emergence phase occurs after emerging of seedlings above soil surface, and is characterised by sudden death of seedlings within a few days (3-5), upon which they topple on the soil surface. Black dot like pycnidia are seen on the necrotic tissue of collar region of seedlings affected with *P. betae*. *P. aphanidermatum* when caused post-emergence damping off, the disease progresses rapidly from root upward while with *P. betae*, the disease extends towards base. *P. betae* occurs more frequently than *P. aphanidermatum* on older seedlings, but once secondary thickening of hypocotyl starts and seedlings become stout, the plants are often recovered. The disease due to *S. rolfsii* appears in patches as a sudden wilting of seedlings followed by yellowing of leaves. The first appearance is marked by drooping of lower leaves followed by scanty white mycelial strands found clinging to the roots. The seedlings are more susceptible at 2 to 4 leaf stage but develop resistance at 6 leaf stage. *R. solani* and *R. bataticola* cause partial damage to roots and stem in hypocotylar region. The collar region of seedlings becomes water-soaked, brown

to black in colour and weakened; plants fall over and die. Mycelial growth of both the fungi can be seen on the surface of affected tissue of severely diseased seedlings. *R. solani* causes higher mortality than *R. bataticola.* Species of *Fusarium* and *Alternaria* incite partial damage to root system leading to yellowing of leaves but most of the seedlings recover after a certain period of infection.

The pathogen *P. betae* is mainly seed-borne in nature and seed borne inoculum plays an important role in recurrence of disease from one season to another, however, soil inoculum also cause infection of sugarbeet seedlings (Mukhopadhyay, 1987; Srivastava and Pandey, 1979). A selective medium for the enumeration and isolation of the pathogen from soil and seed has been developed by Bagbee (1974). Low temperature and cloudy weather favour disease development.

Other seedling pathogens, *P. aphanidermatum*, *R. solani*, *R. bataticola*, *S. rolfsii*, *Fusarium* spp. and *Alternaria* spp. are natural inhabitants which survive as oospore, sclerotia, chlamydospores, micro- and macro-conidia and conidia on dead and decaying crop debris. These fungal structures play an important role for the spread and carry over of the disease from one season to another. Inoculum potential, longevity of fungal structures, interactions with other pathogens, temperature and moisture influence disease severity. Singh and Srivastava (1987), Srivastava and Tripathi (1998b) studied the effect of interactions of various seedling pathogens like *P. aphanidermatum*, *R. solani*, *R. bataticola* and *S. rolfsii* in combination. All these pathogens in combination increased severity of disease complex of sugarbeet.

The management of seedling diseases through fungicides has been achieved by many workers in India and other countries. EMP (ethyl mercury phosphate), Dexon and Thiram seed dressing and soil or row treatment have been found effective against *Pythium* spp. (Ahmadinejad, 1973; Hubbel and Paul, 1993; Pandey and Agnihotri, 1985). Seed treatment with Dexon or Ridomil or Apron (different formulations of Metalaxyl) gives excellent control of damping-off caused *Pythium* spp. (Lamey *et al.* 1993; Mukhopadhyay and Chandra, 1982; Rama, 1980;). Careful water management and seed treatment with Metalaxyl fungicides or Thiram or Hymexazol are efficient in controlling Pythiacecous fungi (Payne and Williams, 1990). The

efficacy of mercurials, PCNB, Demosan (chloroneb), Vitavax (carboxin) in preventing seedling infection due to *R. solani* has been proved (Pandey and Srivastava, 1990; Sen *et al.,* 1974; Srivastava and Tripathi, 1999). The seed treatment with Thiram, Benomyl and carboxin has been found effective in reducing the infection of *R. solani*, thus eliminating the need for hazardous use of mercurials (Srivastava, 2000). The control of *S. rolfsii,* which causes seedling diseases, can be effectively achieved by seed treatment with PCNB or Demosan (Rama, 1980). Seed treatment with benzimidazole fungicides, *viz*., Bavistin and Benlate was found to be most effective in checking seedling infection due to *F. oxysporum* and *P. betae* (Rama, 1981).

Pelleting of seeds with fungicides using methyl cellulose as a sticker improved the efficacy of fungicidal treatment in reducing seedling disorders (Sen, *et al.*, 1974). In recent years, the use of pelleted seeds has increased considerably in almost all sugarbeet growing countries. A successful and suitable technique for pelleting of sugarbeet seeds with various fungicides has been standardised at this Institute, using bentonite clay as base material and methyle cellulose as sticker (Fig. 4). Through use of this technique, seedling mortality due to *P. aphanidermatum* was reduced significantly as compared to conventional seed treatment (Singh *et al.* 1978). Subsequently,
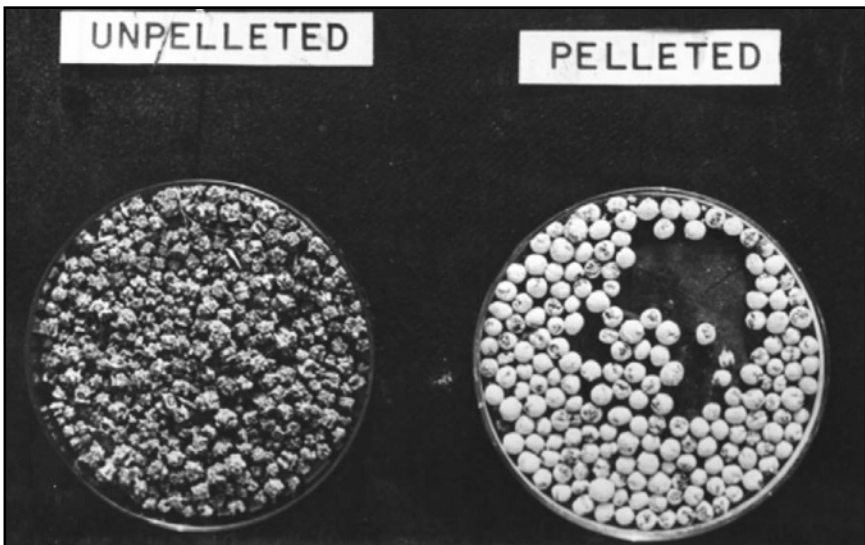
Fig. 4: Unpelleted and pelleted sugarbeet seeds.

pelleting with PCNB effectively reduced the disease caused by *R. solani* and *S. rolfsii* (Singh, *et al.* 1982) and Thiram against seed-borne infection of *P. betae* (Srivastava and Singh, 1984). Pelleting with a mixture of two fungicides like Vitavax + Thiram or Vitavax + PCNB or Bavistin + Thiram (Table 1) provides better protection of seedlings from disease complexes caused by four soil-borne pathogens *viz.*, *P. aphanidermatum*, *R. bataticola*, *R. solani* and *S. rolfsii* (Srivastava and Tripathi, 1998) as compared to conventional seed treatment *i.e.* steeping. The authors view is that the combined effect of fungicides and their sufficient quantity retained at the site where the infection occurs, as well as appropriate concentration of fungitoxicants in the pelleted seed, may be responsible for providing effective and better management of seedling diseases (Fig. 5). Conversely, with conventionally treated seeds, effectiveness of fungicides is lost due to handling, leaching and more exposure to soil.

Fig. 5: Seedling raised from pelleted sugarbeet seed.

TABLE 1

Management of sugarbeet seedling disease complex caused by
soil-borne pathogens through combination of fungicides
(1988-89 and 1989-90).

Treatment	% seedlings Emerged	% seedling mortality	
		Pre-emergence	Post-emergence
I. UNINFESTED SOIL			
A. Unpelleted seed	58.1	-	-
B. Pelleted seed	69.5	-	-
II. INFESTED SOIL			
A. Unpelleted seed			
1. Steeped in water	27.0	43.9	62.5
2. Steeped in :			
i) PCNB+Thiram	27.8	43.8	53.1
ii) Vitavax+Thiram	50.7	18.0	46.8
iii)Vitavax+PCNB	49.9	19.9	35.9
iv)Bavistin+Thiram	50.1	18.0	40.3
v) Bavistin+PCNB	48.6	21.7	35.8
B. Pelleted Seed			
1. Pelleted without fungicides	48.9	32.4	53.7
2. Pelleted with:			
i) PCNB+Thiram	60.8	16.5	33.9
ii) Vitavax+Thiram	68.9	9.0	37.7
iii)Vitavax+PCNB	73.5	9.3	34.0
iv)Bavistin+Thiram	68.4	7.7	26.8
v) Bavistin+PCNB	64.8	12.4	30.2
CD at 5%	7.1	9.25	6.35

Data transformed as Sin-1% seedling emerged/pre-and post mortality

The excellent management of damping-off due to *P. aphanidermatum* has been obtained with integration of soil amendments of *Trichoderma harzianum* and seed treatment with Metalxyl (Mukhopadhyay and Chandra, 1982). Biological control of seedling diseases caused by *Pythium* spp., *R. solani*, *R. bataticola*, *S. rolfsii* and *P. betae* has been demonstrated (Abada, 1994; Mukhopadhyay and Upadhyay, 1983; Perez De Algaba *et al.*, 1992; Srivastava and Tripathi, 1996a). Srivastava and Tripathi in 1996 reported excellent disease control *i.e.* 67.87% *S. rolfsii* when soil application of *T. harazianum* was combined with coating of seeds with the same antagonist. Various strains of *Gliocladium virens,*

Cladorhinum foecundissimum, Pseudomonas fluorescens, Stenotrophomonas maltophilia and *Streptomyces griseoviridis* as bio-agents has been reported effective in reducing seedling diseases caused by *Pythium* spp.; *R. solani*, *P. betae*, *Fusarium* spp. (Dunne *et al.*, 2000; Lewis *et al.*, 1995; Ruppel, 1993; Thrane *et al.*, 2000; Vaughan and Rush, 1993).

Under Indian conditions, crop rotation and other cultural practices give only limited protection from these seedling diseases. However to some extent, timely sowing of crop (first fortnight of November), good soil drainage, moderate irrigation, and avoidance of susceptible hosts are helpful in reducing these diseases. Stout seedlings are less affected by these pathogens. Sowing of multigerm seed should be avoided because during the singling operation seedlings are damaged and become vulnerable to attack by these pathogens. Currently monogerm seeds are in popular usage for sowing in almost all sugarbeet growing countries (Srivastava, 1998).

4.   Foliar Diseases

Among foliar diseases, Cercospora leaf spot is the major disease of sugarbeet followed by Alternaria leaf blight and powdery mildew. Other diseases, *viz.*, Ramularia, Colletotrichum and Phoma leaf spots, are of minor importance. Therefore, in the present communication three diseases of major importance (Cercospora leaf spot, Alternaria leaf blight and powdery mildew) and two diseases of minor importance *i.e.* Colletotrichum leaf spot and Phoma leaf spot have been discussed. Ramularia leaf spot is of rare ocurrence and sporadic in nature.

4.1. Cercospora leaf spot

Cercospora leaf spot (also known as brown spot or leaf blotch), due to *Cercospora beticola,* is one of the most widespread and destructive foliar diseases of sugarbeet. It was first reported from Italy in 1876. Since then, severe epidemics of the disease have been reported in almost all sugarbeet growing countries of the world. In India, it was reported by Mukhopadhyay (1968a) from Pantnagar. It is considered to be most devastating foliar disease of the root crop in the plains and

seed crop in the hills (Mukhopadhyay, 1974). It also occurs in a severe form in cooler regions of Jammu and Kashmir (Kaw *et al.* 1979), the foot hills of Kumaon, Pantnagar (Mukhopadhyay, 1968a) and the plains of Punjab (Sandhu and Bhatti, 1969), Delhi (Juneja *et al.*, 1976) Sundarbans, West Bengal (Srikanta Das, 1990), Sriganganagar, Rajasthan (Rajpurohit and Singh, 1992) and Lucknow (Srivastava and Tripathi, 1996b). Mukhopadhyay and Rao (1978) reported a 33% reduction in root yield and 44% in sugar production. In seed crop, it adversely affects the size and quality of seed.

Considering the devastating nature of the disease, extensive work has been done by many plant pathologists in the country (Mukhopadhyay, 1992).

The symptoms of the disease start appearing on lower and older leaves. At the initial stage, the disease is characterised by the appearance of minute, translucent spots, clearly visible only when the infected leaves are held up to sunlight. Within a few days, the spots turn into diserete circular lesions 3 to 5 mm in diameter (Fig. 6) having necrotic grey centres with reddish brown to black margins. The spots are often surrounded by yellowish to greenish halo. Elongated lesions may also occur on petioles and circular lesions on the crown portion of the root which is not covered by soil. At an advanced stage, the spots may coalesce covering major leaf areas. The affected leaves shrivel, resulting in pre-mature defoliation. Minute greyish dots (pseudostromata) composed of conidia on short conidiopheres are observed on the surface of lesions. These dot-like structures can be easily seen by using a hand held magnifying lens.

The disease is caused by *C. beticola*. The pathogen grows very slowly on artificial media. However, to overcome this problem, a suitable selective medium, *i.e.* sugarbeet leaf extract agar medium (SBLEA) has been developed (Calpouzos and Stallknecht, 1965). Physiologic specialization among the strains of pathogen based mainly on cultural, morphological and physiological characters have been described. Based on these characters, 58 monosporic isolates of *C. beticola* collected from different geographical locations in India were classified into nine biological forms (Pal and Mukhopadhyay, 1984; 1986). Pathogen is known to produce a non-host specific toxins like cercosporin (Balis and Payne, 1971; Milat and Blein, 1995) beticolins (Ducrot *et al.*, 1994; Milat *et al.,* 1993).

Fig. 6: Cercospora leaf spot of sugarbeet.

Primary infection of the plants takes place through mycelia, conidia and stroma (sporodochia) of the pathogen, which gets into soil *via* infected crop debris and infested seeds. These sources also attribute for annual recurrence of the disease. Besides seed-and soil-borne inoculum, other host plants like fodder beet and weed species belonging to genera *Chenopodium, Amaranthus* and *Atriplex* also serve as sources of infection. Secondary infection takes place by conidia produced on infected leaves which are disseminated mainly by wind, but also to some extent by irrigation water, rain splash, dew, insects and mites. Cercospora epidemics are dependent on warm and wet weather. Its incidence is usually high and spreads rapidly during intermittent rains and high humid conditions (optimum RH 92-95%).

The environmental factors temperature, rainfall, wind speed and direction affecting the sporulation of the pathogen on diseased plants, are mainly responsible for the spread of the disease from one location to another (Mukhopadhyay, 1987). Based on data collected on these factors and Cercospora leaf spot incidence from previous years, few simulation models for forecasting of disease epidemics have been developed and reported from Italy (Battilani *et al.,* 1999; Rossi *et al.,* 1994).

Spraying of fungicides has been found very effective for the management of the disease under field conditions. Among the non-systemic fungicides, 4 to 6 sprays of copper compounds or organotin compounds at 15day intervals provide for good control of the disease. Although organotin compounds are superior in action over other protective fungicides, these were withdrawn from commercial production due to health hazards. Systemic fungicides like Benomyl, Carbendazim, Thiobendazole and Thiophanate methyl provide excellent control of this disease. Even one spray of these fungicides at 100 g a.i./ha gave effective control of the disease with increase in all yield parameters (Mukhopadhyay and Bandopadhyay, 1979). However, exclusive and continuous use of these chemicals for three years or more has led to selection or development of benzimidazole resistant strains of *C. beticola* (Pal and Mukhopadhyay, 1985; Weiland and Halloin, 2001). Under these circumstances, other fungicides and alternative measures should be adopted to reduce the infection of *C. beticola*.

Varieties vary in their response to *Cercospora* infection (Mukhopadhyay *et al.*, 1985; Waraitch, 1988; Rajpurohit and Singh, 1992; Srivastava and Tripathi, 1996b). Resistance to pathogen has been found to be correlated with phenolic compounds (hydroxytyramine content of the leaves). Breeding for disease resistance has been fairly successful in many sugarbeet growing countries of the world (Golev *et al.*, 1995; Hayashida *et al.*, 1999; Rossi, 1995; Saunders *et al.*, 2000; Smith, 1985) and a number of varieties showing resistance to Cercospora leaf spot have been developed. The techniques for creating uniform epiphytotics of individual lines have been described (Adams *et al.*, 1995; Naidu and Mukhopadhyay, 1982). Breeding for disease resistance would be useful tool for managing the disease in the country.

Among cultural methods, field sanitation, burning of infected crop debris, a rotation system of 2-3 years with non-host crops, deep ploughing and use of disease free seed would greatly help to reduce the disease in fields. Over all, an integrated approach is recommended for managing the disease economically, involving cultural methods, resistant cultivars, and spraying of fungicides.

4.2. Alternaria leaf blight

Next to Cercospora leaf spot, Alternaria leaf blight is also an important and destructive disease of sugarbeet. It is prevalent in all sugarbeet growing countries of the world. It was first recorded from coastal valleys of California, U.S.A. (Mc-Farlane *et al.*, 1954). In India, it is reported from Pantnagar and Lucknow (Mukhopadhyay, 1969; Singh and Srivastava, 1969). In the country, it has been observed in moderate to severe form from Punjab, Delhi, Sriganganagar, Sunderbans, Jammu and Kashmir and Mukteswar. The disease is caused by two species of *Alternaria*, *i.e. A. alternata* and *A. brassicae.* Out of these two *A. alternata* is more damaging and may destroy upto 30% leaf area.

Leaves are only part to be affected by the disease. Symptoms as small flecks are very rarely seen on petioles. Two distinct symptoms of these two species are seen in the field. The leaf spots produced by *A. alternata* are upto 10 mm in diameter, irregular in shape, dark brown to black in colour and are common on the margins (Fig. 7) where as, the symptoms caused by *A. brassicae* form concentric, zonate, size upto 15 mm in diameter. Under field conditions, the spots are found more frequently on lower and older leaves than on upper and younger leaves which are exposed to full sunlight. As the disease progresses, the spots increase in size and become dark brown or black in colour with water soaked margin. The spots may appear on any portion of lamina. Water-soaked, sub circular brown spots with necrotic flecks is the centre appear in both surfaces of leaves. The colour of central necrotic lesions is lighter. The spots may coalesce with each other. Marginal infection of leaves causes drying and upward curling of edges. In an advanced stage of disease development, the central necrotic lesions get dried and fall off, resulting in shot holes. Blackish fungal growth, on which conidia are borne, often covers

Fig. 7: Alternaria leaf spot of sugarbeet.

necrotic lesions under humid conditions. The author observed a lot of variability among the symptoms during the survey of disease at different locations. Other species of *Alternaria* or other pathogens may be associated with the disease.

In Indian conditions, the disease starts appearing in the fields from January onward. The pathogens have a wide host range including other members of family Chenopodiceae. Primary infection takes place through wind-borne conidia transmitted through the infection of other hosts. The infested seeds obtained from infected crops serve as the basic source of inoculum. The pathogen survive in field on infected crop debris and the secondary infection takes place by wind borne conidia derived from the sporulation of a diseased portion of the same

host. The role of rains and wind is most important in the transmission of disease from one location to another. Temperature, humid weather with high atmospheric humidity, dense mist, fog and dew are of great importance for the development of disease in the fields. A high incidence of disease has been observed with in the temperature range of 25-30 ºC.

The disease is partially managed by spraying of Dithane M-45, kasumin or Brestanol (Agnihotri *et al.*, 1972). Out of these, two or three sprays of Dithane M-45 @ 2.5 kg/ha per spray at 15-day intervals before the appearance of the disease gives effective control. The disease was also controlled by spraying of copper fungicides and Captan. Breeding for disease resistance has been attempted in many countries but it has not been found successful due to the very wide host range of the pathogen.

4.3. Powdery mildew

Powdery mildew caused by *Erysiphe betae* is also a serious disease of sugarbeet. It is prevalent in arid climates of the Middle East, Russia, Europe, U.K., U.S.A. and Canada. The disease is recorded from all over India (Karve, 1972; Mukhopadhyay, 1968; Singh *et al.*, 1971). The disease was observed in a severe form in the Phalton area of Maharashtra under warm and dry weather conditions (Karve, 1972). The disease reduced the root yield by 20-25% and has been found to be the main cause for low production of sugar in Maharashtra (Karve, *et al.*, 1973).

The disease appears first on lower and older leaves and gradually spreads towards the upper and younger leaves. It is characterised by the formation of white, later grey, tan mildew areas on both sides of the leaf (Fig. 8). In general, infection is more common on upper surface of leaves. In advanced stage of disease development, mildew patches enlarge and coalesce and leaf looks as if dusted with white powder. The superficial mass consists of mycelia and spores of the pathogen. Severely affected leaves turn yellow and ultimately dry up. Sometimes the telomorph stage of the pathogen has been observed on the surface of leaves as minute, spherical, orange-brown to black fruiting bodies (cleistothecia) embedded in the fungal hyphae.

Fig. 8:   Powdery mildew of sugarbeet.

The disease is caused by a fungus *Erysiphe betae* (Syn. *E. polygoni, E. communis, Oidium erysiphoides, Microsphaera betae*). It is an obligate parasite and can not be cultured on artificial nutrient media. The fungus grows entirely on the host surface except the haustoria which penetrate the epidermal cells and absorb nutrients for its growth. However, the critical stages in the development of pathogen on sugarbeet leaf from the germination of conidia to the establishment of haustorium on the epidermal cells of the host has been reported (Mukhopadhyay and Russell, 1979a). Primary infection of the disease takes place through ascospores produced on sugarbeet plants and other weed hosts infected during proceeding season. Secondary spread/ infection takes place through the large number of conidia produced

during primary infection. The disease is favoured by hot and dry weather and develops best in cool nights and warm days.

An integrated approach involving destruction of crop debris to destroy the surviving structures (cleistothecia), spraying of fungicides and host resistance should be recommended for managing the disease effectively. Disease control has been exclusively achieved by spraying of fungicides like wettable sulphur and other sulphur formulations (Karve *et al.*, 1973; Russell and Mukhopadhyay, 1981; Cicco and Curtis, 1993). Besides, many systemic and other protective fungicides also provide effective management of the disease (Russell and Mukhopadhyay, 1981; Cicco and Curtis, 1993; Asher, 2000). For spraying of fungitoxicants, the first sign of infection on any part of the leaves is very critical and important. The two weeks delay in spraying with sulphur decreased sugar production by 17% in California, U.S.A. (Hills *et al.*, 1975). No disease control was achieved when the chemicals were sprayed after 50% of the plants are already infected. Breeding for resistant/tolerant varieties against the disease has been found fairly successful in many sugarbeet growing countries of the world. Though, most of the commercially grown cultivars are susceptible to powdery mildew infection. Therefore, the genetics of host resistance and virulence of the pathogen have to be studied extensively to develop varieties resistant to powdery mildew. The mechanism of disease resistance on a number of varieties has been studied, and some varieties more resistant/tolerant to powdery mildew infection have been reported (Mukhopadhyay and Russell, 1979b; Luterbacher *et al.*, 2000; Lewellen, 2000). Recently in California, inheritance of powdery mildew resistance has been identified in two wild beet sources *i.e. Beta vulgaris* sub sp. *maritima* accessions WB 97 and WB 242 and these sources are being used to develop resistant varieties against powdery mildew (Lewellen and Schrandi, 2001).

4.4. Colletotrichum leaf spot

Colletotrichum capsici inciting leaf spot of sugarbeet is a less known disease (Singh *et al.,* 1974). The disease is of rare occurrence and thereby indicates only insignificant loss to the crop. Both mature and young leaves affected by the disease are recognized by the appearance of circular to oval, greyish brown to dark brown spots. The size of

Fig. 9: Colletotrichum leaf spot of sugarbeet.

spots increases with age and varies from 1-5 mm mostly 2.5 to 4.0 mm in diameter (Fig. 9). In an advanced stage of disease development, spots coalesce. Symptoms appear both in the margins as well as on the lamina of leaves. The diseased tissue dries up and collapses, causing depression in the centre of spots. Acervuli usually develop on the affected areas in concentric rings. The knowledge regarding the disease is rather rudimentary. Therefore, intensive studies on the epidemiology and management are needed.

4.5. Phoma leaf spot

Phoma betae causing leaf spot infection was first described by Oudemans in 1877 as *Phyllostica betae*. Later on its name was changed

to *Phoma betae*. In India, the disease is of rare occurrence and reported from Kashmir valley (Singh *et al.*, 1973), Delhi (Mukerji and Bhasin, 1986). Later on it was also observed many times from Lucknow, Mukteswar and other places. Primarily it is a serious seedling pathogen and minor leaf spot pathogen. The pathogen is sporadic in nature and the disease does not cause any substantial damage.

Infection of leaves occurs in root and seed crop but is confined mostly to mature and older leaves. The pathogen produces dark brown, circular to oval spots, 10-20 mm in diameter on the upper surface of leaves and have poorly defined margins (Fig. 10). Within the necrotic area of lesions, concentric dark brown rings occur near the perimeter in which minute, spherical, black pycnidia develop. These structures can be seen by the naked eye. In the presence of moisture, the pycnidia

Fig. 10: Phoma leaf spot of sugarbeet.

exude a gelatinous mass of spores which are scattered by rain drops or wind. The teleomorph stage (*Pleospora bjoerlingii*) of the pathogen has not been recorded in India.

5.   Root Diseases

Adult and mature roots of sugarbeet plants are affected by a number of fungal pathogens causing various types of root rots. Among these, Sclerotial root rot (*S. rolfsii*) is the most destructive disease causing about 50% damage of the roots under favourable conditions. Other root rots, of the like dry root rot (*R. solani*) and charcoal root rot (*R. bataticola*) may cause 15-30% destruction. Both yield and sucrose in the root are adversely affected. Rhizopus root rot (*R. oryzae*) and Fusarium root rot (*F. chlamydosporum*) are of minor importance and sporadic in nature.

5.1. Sclerotial root rot

The sclerotial root rot due to *Sclerotium rolfsii* commonly known as "Southern stem and root rot" is of great economic importance causing much damage in the tropics and sub-tropics. However, crop losses are greater in the tropics than in the sub-tropics. The disease is a limiting factor in the cultivation of sugarbeet crop in Southern U.S.A., in warmer, humid areas of Europe, Middle east, India and Asia. In India, the malady was first observed in 1965 (Singh, 1965). Since the introduction of the crop in the country, the disease has become a potential threat to successful cultivation of sugarbeet. It causes 14-59% loss in root yield and reduces sugar content up to 20% in certain varieties under favourable conditions (Mukhopadhayay, 1971; Sharma and Pathak, 1994; Waraitch *et al.*, 1986).

Under Indian conditions, the disease appears during March. The first visible symptoms in the field include yellowing and wilting of leaves followed by rotting of roots of affected plants. White cottony mycelium develops on rotted basal portions of roots and causes gradual semi-watery decay. As the mycelial growth advances, the affected leaves turn yellow and wither pre-maturely. At later stage mycelial growth becomes more profuse and almost covers the major portions

of the fleshy root. Decomposition gives a distorted appearance to the roots. Such affected roots become unfit for sugar extraction as well as feeding animals. On rotted roots, innumerable small, light to dark brown sclerotia of mustard seed size develop on the mycelium (Fig. 11). These hyphal strands and sclerotia are also found in the soil, radiating outwards from affected roots. Such affected plants topple down on the ground. The diseased plants can easily be pulled out due to massive damage to the tap root system as a result of rotting. The fungus also causes seedling blight of sugarbeet resulting in a poor stand of crop.

The disease is caused by fungus *Sclerotium rolfsii* (imperfect stage). The perfect stage is also known as *Pellicularia rolfsii* (Syn. *Corticium rolfsii, Athelia rolfsii*). The imperfect stage consists of
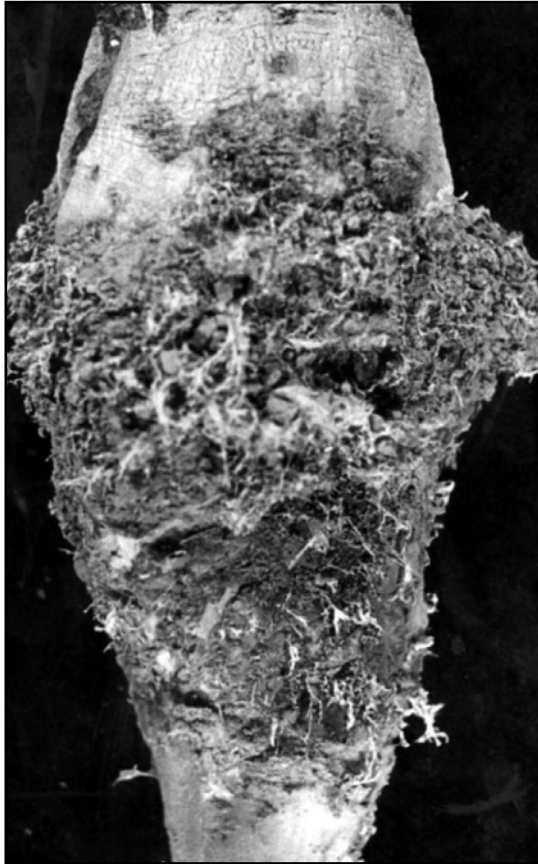
Fig. 11: Sclerotial root rot of sugarbeet.

mycelium and sclerotia. It can be easily grown on potato dextrose agar (PDA) medium under laboratory conditions. A medium for selective isolation of the fungus from soil has been developed and reported (Backman and Rodriguez-Kabana, 1972).

The pathogen penetrates non-wounded seedlings directly by the formation of appressoria. Penetration may also take place through natural openings such as lenticels and stomata. The mycelium is both inter- and intracellular. It produces both pectinolytic and cellulolytic enzymes both in culture and host tissue and also some metabolites *viz.*, oxalic acid.

The fungus survives in soil from one season to another by means of sclerotia formed abundantly on affected roots, crop debris, adjoining soil and other suitable substrates (Fig. 12). Under favourable

Fig. 12: Survival of sclerotia of *S. rolfsii* on crop debris of sugarbeet.

conditions, these sclerotia germinate and give rise to vegetative mycelium and a pathogenic phase. The fungal mycelium first grow near roots and form a network of strands in surrounding soil. As the strands extend through soil, they infect healthy roots and continue their destruction.

In the plains of India, the crop is usually grown on ridges and this practice is likely to stimulate the disease infection because the lower leaves of adult plants invariably remain in touch with the soil. Such leaves pick up infection quickly from soil providing an ideal medium for pathogenesis. Close spacing between plants facilitates the secondary spread of the disease in sugarbeet.

Sclerotia are the principal means of survival of *S. rolfsii* in soil even in the absence of suitable hosts or conditions favouring its active growth. Thus sclerotia which persist for long periods in soil serve as the source of primary infection. These are spread *via* cultivation and irrigation water for secondary infection from one location to another (Duffus and Ruppel, 1993). Disease severity is influenced by the population of viable sclerotia (inoculum density) in the beet field and the longevity of sclerotia in soil. Sen *et al.*, (1979) reported that inoculation of sugarbeet in first week of February using 750g of sand maize-meal inoculum causes the highest and most uniform mortality of roots. Temperature influences root rot incidence of sugarbeet. The maximum disease development occurs at temperatures approximately favourable for the growth of the pathogen in culture, *i.e.* 30-35°C. Disease incidence and severity gradually reduces as the temperature decreases. Minimum disease severity was noted at or below 15 °C. Moisture also influences the root rot development. It has been observed that fields receiving 16 irrigations during crop season show minimum root rot incidence and maximum root yield (Table 2) as compared to 12, 8 and 4 irrigations, respectively (Singh *et al.*, 1986). Similar observations have also been recorded by Maiti *et al.* (2000). Saturated soil moisture conditions at higher frequency level of irrigations may cause lysis of hyphae and sclerotia of *S. rolfsii* which in turn reduces disease incidence. The disease occurs in many types of soil, but it is often severe on light sandy soils followed by sandy loam or loam soils (Mukhopadhyay, 1987; Srivastava, 2000).

TABLE 2

Effect of number of irrigations on disease incidence and
root yield of sugarbeet

Irrigation level (Number)	Disease incidence (in%)	Root yield (t/ha)
4	41.5	21.6
8	33.3	34.4
12	32.5	36.4
16	27.8	49.0
CD at 5 per cent	4.6	8.7

For the management of the disease, no single control is effective. Prevention of the disease is far more important and better than to check it when the plants have been infected in field. Therefore, integrated disease management (IDM) system involving cultural, chemical, biological and host resistance may be employed to manage the disease.

Sanitary measures like uprooting and burning of diseased plants, particularly at early stage of infection should be followed. After the harvesting of the crop, all the diseased plants also along with crop debris of other plants should be burnt completely in the field. Avoidance of sugarbeet sowing in infected fields can minimise the effect of disease on yield. It is desirable to determine the distribution and intensity of infestation of *S. rolfsii* in the field before sowing, for which various techniques have been reported (Punja *et al.*, 1985; Srivastava *et al.*, 1987). Under Indian conditions, early drilling of sugarbeet *i.e.* in first half of November reduces the root rot appreciably and also increases rot yield. Crop rotation is not very effective since the pathogen has a wide host range. However, rotation with less susceptible crops like maize or wheat may result in less disease incidence in subsequent years by lowering the inoculum levels.

Nitrogenous fertilisers, like calcium nitrate, urea, calcium, and ammonium sulphate have been found effective in reducing root rot incidence under Pantnagar conditions (Mukhopadhyay, 1987). The effect of nitrogen has been attributed partly due to stimulation of a biological control agent, *Trichoderma harzianum* (Mukhopadhyay and Upadhyay, 1981). Analogous trials conducted at IISR, have not confirmed above observations. Potassium application had no significant

effect on root rot incidence (Singh *et al.*, 1986). Groundnut and msustard oil cakes at 50q/ha significantly reduced root rot incidence and also increased root yield when applied 15 days prior to planting (Table 3). Oil cakes stimulate the population of micro-organism antagonistic to *S. rolfsii* (Sen *et al.*, 1972).

TABLE 3
Effect of soil amendments on disease incidence and
root yield of sugarbeet

Amendments	Dose (q/ha)	Disease incidence* (%)	Root yield* (t/ha)
Groundnut cake	25	23.92b	29.62bcd
	50	17.10b	43.37a
Neem cake	25	39.38a	23.30cd
	50	43.65a	18.50d
Mustard cake	25	24.61b	33.25abc
	50	21.34b	36.40ab
Control	-	29.41a	23.25cd

*Means followed by different letters in column are significantly different at 1% level according to Duncan's multiple range.

Biological control through *Trichoderma harzianum* and *T. viride* have been found effective in reducing root rot incidence both under glass house and field conditions (Ciccarese, *et al.*, 1992; Mukhopadhyay and Upadhyay, 1983; Srivastava and Tripathi, 1996a; Upadhyay and Mukhopadhyay, 1986). Soil application of *Trichoderma harzianum* combined with seed pelleting with biological agent gave effective control of seedling as well as root rot of adult sugarbeet plant (Srivastava and Tripathi, 1996a).

Several fungicides, like carboxin (Vitavax), benomyl (Benlate), quintozine, Demosan (chloroneb), Dithane M-45, Calixin, Bavisitn, Thiram and Brassicol (PCNB) have been evaluated as soil drench to manage the sclerotial root rot in fields (Mukhopadhyay and Thakur, 1971; Sharma *et al.*, 1990; Singh *et al.*, 1974; Waraitch *et al.*, 1986). Ridge soil drenching with PCNB or Demosan (15 kg/ha) or Vitavax (2 kg/ha) during mid February minimised the disease and also increased

root yield (Mukhopadhyay and Thakur 1971). Soil drenching with PCNB or Demosan at 15 or 20 kg/ha (Table 4) significantly reduced the disease and improved the yield (Singh *et al.*, 1974).

TABLE 4

Effect of different fungicides on incidence and root yield of sugarbeet

Fungicide	Dose (q/ha)	Disease incidence* (%)	Root yield* (t/ha)
Bavistin	15	20.43	39.10
	20	20.04	40.20
Demosan	15	16.85	44.37
	20	14.86	51.87
Brestanol	15	8.74	47.12
	20	8.80	47.91
Pentachloronitro benzene	15	5.44	51.66
	20	2.13	47.29
Control	—	24.13	43.12
CD at 5%	—	6.62	8.00

*Means followed by different letters in column are significantly different at 1% level according to Duncan's multiple range.

Sharma *et al.* (1990) reported that PCNB (10 kg/ha) as soil drench controlled the disease and slightly improved the sucrose content. Combined application of mustard or groundnut cakes at 50 q/ha (15 days prior to sowing) and PCNB at 15 kg/ha (as soil drench in mid-February) also reduced the incidence of root rot (Sen *et al.*, 1973). Besides fungicides, various insecticides, nematicides and weedicides have also been found effective in reducing sclerotial root rot to some extent under field conditions. Although few of these chemicals have shown promise against the pathogen under field conditions, the use of these chemicals on a large scale are not economically feasible in India due to their high costs. Two effective fungicides, PCNB and Brestanol, have been banned from use in the country due to health hazards. Therefore, the emphasis should be placed on working out an alternative to effective fungitoxicants.

Due to the extremely wide host range of *S. rolfsii*, host resistance has not become a viable control measure for this pathogen. In India, a number of sugarbeet genotypes/cultivars have been screened through a regular screening programme. Under favourable environmental

conditions, all sugarbeet varieties display susceptibility to the pathogen, however, the degree of susceptibility varies among the varieties (Srivastava *et al.*, 1993; Srivastava *et al.*, 1994). A few indigenously developed genotypes, like IISR-2, LS-6 and IISR Comp-1, show comparatively low incidence of the disease. Therefore, these genotypes may be exploited further for breeding tolerant genotypes for commercial cultivation in disease prone areas in Indian conditions.

5.2. Dry root rot

Dry root rot caused by *Rhizoctonia solani* also known as "Rhizoctonia Root or Crown Rot" or "Dry Root Rot Canker" has been reported from most of the temperate, tropical and sub-tropical countries. It is the most serious root disease of sugarbeet in the USA and Europe and predominantly occurs in hot climates. The disease was first described by Le Clerg (1939) as Dry Root Rot Canker. In India it was recorded in 1971 (Singh *et al.*, 1971, 1974). The disease is prevalent in all sugarbeet growing areas of the country and about 15% of the roots are damaged in the fields.

In the field, the disease is characterised by a greyish brown to reddish-brown discolouration of mature roots around the bases of lateral roots. Diseased roots show a woody appearance and concentric rings develop on the infected portion. The lesions are slightly sunken and beneath there, pockets or deep cankers of dirty brown spongy decayed tissue develop which are sometimes filled with fungal mycelium. Affected areas are clearly delineated from healthy portions of roots by formation of a cinammon brown zone. In several instances with the advancement of the disease, entire affected roots disintegrate, exposing the fibro-vascular strands. The leaves of diseased roots wither away gradually and black lesions appear on the bases of petioles. Such leaves collapse and die but remain attached to crown, forming a rossette of brown leaves (Fig. 13). The pathogen also causes seedling damping-off and under humid conditions, certain strains can cause a leaf blight.

The pathogen, *R. solani* (Syn. *Corticium solani, Hypochnus solani, R. practicola*; teleomorph stage, *Thanatephorus cucumeris*) grows well in culture media at temperatures ranging from 24-28°C. It produces mycelium, sclerotia and chlamydospores in culture.

Fig. 13: Dry root rot of sugarbeet.

Sometimes these sclerotia are also found on or in decaying tissues which may vary widely in shape and size. The inconspicuous teleomorph stage (*T. cucumeris*) is saprophytic in nature and has rarely been found on sugarbeet. However, the role of basidial stage producing foliage/petiole blight has been established (Naito and Sugimoto, 1980; Windels *et al.*, 1997). Strain differences among the isolates of *R. solani* obtained from moribund seedlings, diseased mature roots and blighted leaves have been observed. However, the species is subdivided in different anastomosis groups (AGs) based on the ability of isolates to fuse with one another cytoplasmically. A PCR (Polymerase Chain Reaction) based method for differentiation of *R. solani* anastomosis groups has been reported (Fisher and Gerik, 1993).

The fungal structures (hyphae, chlamydospores and sclerotia, mostly sclerotia) survive on organic debris in soil for longer periods and become active when soil temperature ranges from 25-33 ºC. These fungal structures may also survive in soil in the absence of its host for considerably longer periods (years) by competitive colonization of soil organic matter. Ko and Hora (1971) have developed a selective medium for assessing the population of *R. solani* in soil. The sclerotia germinate in damp weather by producing new mycelial threads which can grow upto 7-10 cm if the food is that distant. Damping-off seedling blight occurs if sugarbeet is sown in warm soil and infection may go on petioles, crown in roots of older plants when soil temperature increases. The pathogen is disseminated through wind, irrigation water or transport of contaminated soil to uninfested fields. Disease severity is influenced by kind of inoculum, amount of inoculum and inoculum density.

For the management of the disease, crop rotation (3-5 years) and other cultural practices like deep ploughing and destruction of diseased plants give substantial protection against dry root rot. Severe root rot occurs with monoculture of sugarbeet; therefore, it should be avoided. Among the many fungicides recommended as soil treatment, only PCNB, as pre-sown soil drench and crown spray application, has been found effective to minimise the disease. Substantial gains in resistance of sugarbeet to Rhizoctonia root rot have been achieved and a number of resistant germplasms were developed and released in many sugarbeet growing countries of World (Benker, 2000; Halloin *et al.*, 2000; Hecker and Ruppel, 1988; Panella and Ruppel, 1996; Scholten *et al.*, 2001).

5.3. Charcoal root rot

Charcoal root rot (*Rhizoctonia bataticola*) is another important disease prevalent both in tropical and subtropical countries. It is reported all over India (Singh *et al.*, 1971, 1973, 1974; Srivastava *et al.*, 1986). About 25% of the roots are destroyed by the pathogen under favourable conditions.

Charcoal root rot appears normally in the month of March *i.e.* about four months after sowing, and its incidence may increase in the month of April. The disease appears on the upper portions of roots and bases

of petioles, and is characterised by a brownish-black discolouration of the crown portion of the root. In advanced stages of disease development, innumerable black sclerotia are produced and affected tissues turn black with a silvery sheen and underlying black, dry decay of roots starts (Fig. 14). In severe infections dry rotting of whole roots occurs and the affected root is reduced to dry mass, shrivels and becomes mummified, giving a charcoal appearance covered with a thin dry papery shell. Leaves and petioles display wilt symptoms, soon turn brown, dies and fall on the ground. Sometimes the pycnidial stage (*Macrophomina phaseolina*) has been observed on the crown portion of the affected root and bases of petioles.

The disease is caused by *R. bataticola*. The pathogen grows well in culture and produces mycelium, sclerotia and chlamydospores. The pycnidial stage is called *M. phaseolina* (Syn. *M. phaseoli*) which produces pycnidia and pycnidiospores. Strain differences among the isolates of pathogen have been reported. A selective medium has been developed by Meyer *et al.* (1973).

Fig. 14: Charcoal root rot of sugarbeet.

Sometimes these structures, particularly sclerotia, are also produced on decomposing roots and leaves in soil, which in turn help the disposal of the fungus and contribute inoculum reservior. Sclerotial bodies contribute towards long term survival. Pycnidiospores have a short life span. These infective propagules germinate in soil in the presence of root exudates and cause infection. The disease is associated with high temperatures. Maximum disease incidence is around 35 to 40°C. The pathogen has a wide host range affecting more than 300 host plants.

The disease may be effectively minimised by soil drenching of PCNB (Srivastava *et al.*, 1986) and through proper manipulations of cultural practices like changing of cropping pattern with avoiding crop rotation with susceptible crops (Singh *et al.*, 1973).

5.4. Rhizopus root rot

Several species of *Rhizopus, viz., R.oryzae, R. arrhizus* and *R. nigricans* are reported to cause root rot of sugarbeet in the U.S.A., Canada and India. Root rot due to *R. oryzae* was first recorded in India by Srivastava and Misra (1972).

In nature, the disease starts as soft water rot and progresses rapidly into healthy tissue. Later, it covers the entire root surface with effuse fungal growth. Complete rooting of roots takes place with in 10-15 days. The diseased tissues turn yellowish brown, become spongy and emit a peculiar repulsive odour (Fig. 15). In field, excessive soil

Fig. 15: Rhizopus root rot of sugarbeet.

moisture and increase in atmospheric temperature (25-30 °C) have been observed to enhance the disease. The disease is sporadic in nature and does not cause any appreciable loss.

5.5. Fusarium root rot

Fusarium root rot also known as Fusarium yellows in many countries, is a disease of minor importance prevalent only in localised areas of the western U.S.A., Germany, Belgium, Netherland and India. In India, two species of *Fusarium* causing root rot in mature sugarbeet plants, have been reported *viz., F. oxysporum* sp. *betae* associated with root crop (Mukhopadhyay and Thakur, 1970) and *F. chlamydosporum* associated with seed crop (Srivastava *et al.*, 1999) Both the species also cause "seedling wilt" and stalk blight. Additionally two other species *F. moniliforme* and *F. avenaceum* cause only seedling damping-off (Mukhopadhyay, 1987).

  Initial symptoms of the disease include interveinal yellowing of older and mature leaves. As the disease progresses, younger leaves may also show yellowing, and the chlorotic areas thus developed in older leaves may show no external symptoms at intial stage. But, if root is cut through, the greyish to reddish-brown discolouration and rot of vascular system is evident. By gradually cutting away a diseased root from crown to tip, the path of fungus invasion can be traced back to its starting point in a lateral root. It seems that the pathogen enters through small lateral roots rather than wounds. In severe cases, the roots become completely wilted, shrivelled, and finally disintegrate, exposing the fibrovascular strands. The leaves of such plants collapse and dry up completely. Sometimes, the profuse growth of both the Fusaria spp. can be seen on the surface of affected tissue. The growth *F. chlamydosporum* is more consipicuous due to its typical red colour. The fungi produce micro-macro-conidia and chlamydospores which survive in soil and infect root debris. Chlamydospores act as resting structures in soil. Oversummering of these structures in plant debris in soil provides the primary inoculum for the next infection cycle. The disease is favoured by high temperature. Rotation for two years (4 to 5) with non-susceptible crops like cereals and alfalfa has been found to be quite useful for minimising the disease to some extent.

5.6. Root Knot Nematodes/Fungus-Nematode Complex

Nematodes often pose serious problems both in seed and root crops of sugarbeet. Two species of root knot nematodes, *Meloidogyne incognita* and *M. javanica* associated with both seed and root crop have been reported from India (Singh and Misra, 1970). Both species, individually or in association cause severe damage by reducing the yield as well as the sucrose content of roots (Rashid *et al.*, 1981; Singh and Misra, 1974). In general, root knot nematode-affected plants are stunted and there is loss of chlorophyll in the leaves. These two species form galls on the roots (Fig. 16) and can be easily differentiated on the basis of symptoms produced by these galls. *M. javanica* forms small galls on secondary roots while *M. incognita* produces pearl-sized or even bigger galls mostly on primary roots.
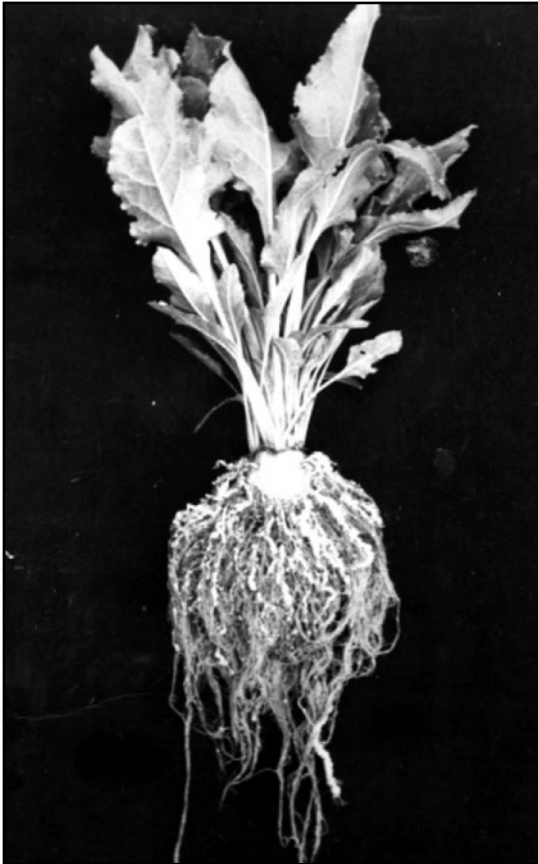
Fig. 16: Sugarbeet root showing root knot nematodes.

Several fungus-nematode complexes have been observed in diseased sugarbeet material collected from different parts of the country. The association of *Pythium aphanidermatum P. ultimum*, *Rhizoctonia solani* and *R. bataticola* with both the species of *Meloidogyne* has been frequently observed (Pandey, 1984; Singh *et al*., 1975). The root knot nematodes in combination with these soil borne pathogens reduce the growth of seedlings as well as mature roots and the effect is additive and severe when two or more pathogens are inoculated together.

Soil fumigation with many nematicides like DD and vapam has given encouraging results in reducing the population of these nematodes. Besides, many other cultural practices like crop rotation, flooding, removal of infected plants, desiccation and growing of antagonistic plants like *Tagetus* (Marigold), *Chrysanthemum* spp., castor bean (*Ricinus communis*) etc. may also be employed to minimise the population of root knot nematodes.

6.   Non-Pathogenic Diseases

Among non-pathogenic disorders boron deficiency or Heart rot and Strangles" are of great economic importance while other diseases like 2,4D injury, Tip burn are of rare occurrence and minor importance.

6.1. Boron Deficiency or Heart rot

Boron is one of the most important trace elements required for the growth of sugarbeet plants. Without its adequate supply, the yield and quality of the sugarbeets are severely depressed. The shortage of this element causes typical symptoms in leaves, petioles, crowns and roots. Boron deficiency has been described as a cause of "Heart Rot" (Bradenberg, 1931). A full description of detailed symptoms has been described by Draycott (1972). The disease is wide-spread, throughout all sugarbeet-growing regions of the world, and symptoms described are similar in all countries. The disease is of common occurrence in all the sugarbeet-growing regions of India (Singh, 1965). The disease usually occurs in patches and losses are difficult to assess.

The disease in the field is characterised by soft rot of young leaves which progresses into roots, turning tissues black. In severe infections, blackish brown dead areas develop on the inner surface of petioles and death of growing point and youngest leaves occurs. Occasionally ladder-like markings are observed on the midrib of leaves. The disease results in formation of cavities of varying sizes in the affected roots which when cut longitudinally, show greyish brown discolouration with grey-coloured streaks between vascular tissues (Fig. 17).

Boron deficiency can be corrected by soil application of borax (32 kg/ha) or foliar application of borax 16 kg/ha in the form of sodium borate (Na3BO3). Treatments need to be repeated each year where sugarbeet is grown (*see* Chatarjee and Dube, this book).

Fig. 17: Boron deficiency of "Heart Rot" of sugarbeet.

3.2. "Strangles"

The disease strangles was reported earlier in the U.K. (Boyd, 1966) and Austria (Krexner, 1967), and was later recorded in India in 1972 at Sriganganagar (Singh, *et al.*, 1973) in the Maribo Resistapoly variety and in many other varieties from other locations. Sometimes 25 to 50% of the roots were found to be affected with the disease.

Vigorously growing plants are prone to break off near the soil level due to high wind velocity or during cultural operations. Upon removal, the roots show constriction at the soil level. The lower part of the root and foliage appear normal. Few such roots also show typical cut worm (*Agrotis* spp.) damage and secondary infection by some species of *Alternaria* and *Rhizoctonia*. In "Strangles" the seedlings are apparently injured by cut worms and seedling pathogens. Subsequently, the tissues above and below the injured ones continue to grow and increase in diameter, with the constrictions remaining in-between (Fig. 18). The two parts of the plants are linked by a narrow
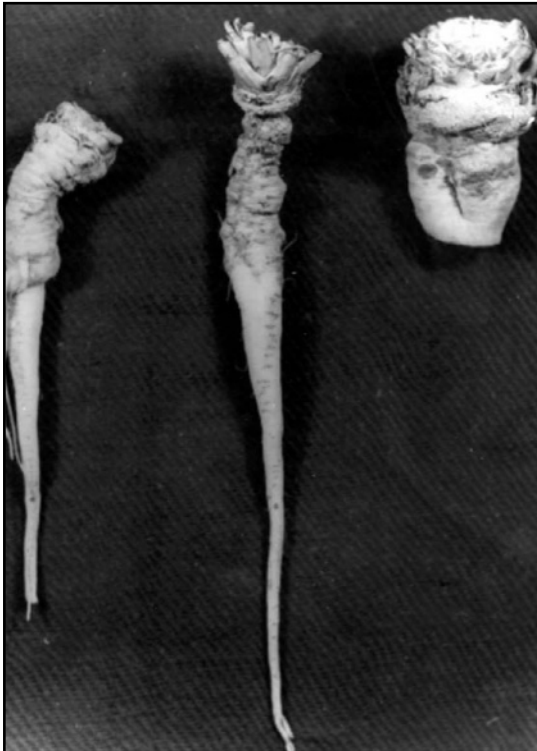
Fig. 18: Strangle disease of sugarbeet.

vascular strip which progressively weakens with the plant growth and roots are easily detopped due to high wind.

The incidence of "strangles" can be reduced by maintaining the level of seed beds with good tilth, low seedling rates and not exposing the plants to much during singling (Mukhopadhyay, 1987).

6.3. Tip burn or calcium deficiency

The disease is of rare occurrence and minor importance (Mukhopadhyay and Thakur, 1978). The sugarbeet plants, in patches, show peculiar symptoms of calcium deficiency or tip burn. The older leaves of plant appear normal but the young leaves a especially the inner ones, fail to develop properly. They consist merely of stalks with very little lamina, develop necrosis and curl inwards. In severe cases, the younger leaves are completely destroyed and the symptoms may be confused with "Heart rot". Defoliation of younger leaves results in development of hollow crowns. The plants display stunted growth and older leaves show puckerings. Calcium deficiency is rarely important economically except in acidic soils which are very unfavourable for sugarbeet cultivation.

6.4. 2,4-D injury

Sometimes when 2, 4-D (2,4-dichlorophenoxy acetic acid) is used as selective herbicide for wheat during December-January, some injury symptoms have been observed. This type of injury symptoms was first reported by Mukhopadhyay and Thakur (1978) from Pantnagar. The sugarbeet plants adjacent to these plots show similar abnormal symptoms. The younger plants become pale, the petiole and hypocotyl elongate, curl and become prostrate. Such plants on subsequent growth give rise to leaves with frilly lamina and produce 3 or more mid ribs instead of single mid-rib. In exceptional cases, the midrib branches produce two or three leaf-like structures. Affected plants are stunted and have poor root symptoms. In later stages, slight recovery of a few plants has been observed.

7.　Conclusion and future strategy:

Although at present the sugarbeet crop is out of commercial cultivation in India, it is still in the protocol of Agricultural Research and has potential as a supplementary sugar crop. It may come up and develop at any time in future. Therefore, greater and concerted efforts will have to made to solve some of the intricate problems, particularly those pertaining to seedling diseases, important leaf spots and root rots. Since no single method of disease control is effective with certain diseases, there is urgent need for intensive experimentation for evolving an integrated disease management (IDM) system involving indigenous fungicides, bio-agents, disease resistant varieties and cultural practices.

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Threat to Vegetable Production by Diamondback Moth and its Management Strategies

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ABSTRACT: Diamondback moth (DBM) *Plutella xylostella* (Linneaus) is one of the most destructive pests of crucifers *viz.,* cabbage, cauliflower radish, knol khol, turnip, beet root, mustard and rape seed in India. The loss in yield caused by the pest varies from 31-100%. The detailed life history as studied by various authors is presented. The pest incidence is generally more during February to September, though it is noticed throughout the year. Population of four or more medium sized larvae (3rd and 4th instars) could render a seedling un-transplantable. One standard hole of size 0.5 to 1.00 cm in diameter is taken as the visual damage threshold for insecticide application. The list of natural enemies recorded on DBM is summarized. The management of DBM through cultural, botanical, IGR's, microbial and chemical methods is discussed. DBM is the first crop pest in the world to develop resistance to DDT and *Bacillus thuringiensis.* The reported cases of resistance development to insecticides and various components of integrated resistance management is summarized. Integrated management of DBM using Indian mustard as trap crop is most promising. This involves planting of paired mustard rows for every 25 rows of cabbage and two sprays with 0.05% cartap hydrochloride to manage the pest on the main crop and spraying the trap crop with 0.1% dichlorvos at 10 to 15 days interval.

1. Introduction

Plutella xylostella (Linnaeus) (Lepidoptera: Yponomuetidae) commonly called Diamondback moth (DBM) is believed to have originated in the Mediterranean area, where most of the cruciferous crop plants have originated. But, now it enjoys a world wide distribution. It is one of the most destructive pests of cruciferous vegetables in the world. At least 128 countries have reported the occurrence of this pest (Salinas, 1986). In India, it was first reported

by Fletcher (1914) and now it is distributed all over India where crucifers are grown, causing yield loss varying from 31 to 100%. Although several control measures *viz*., cultural: Inter-cropping, sprinkler irrigation, trap cropping, crop rotation and clean cultivation; plant resistance, sex pheromones, chemicals, bio-control agents have been tried, none of these have given satisfactory results (Talekar and Shelton, 1993).

2.   Economic status

The loss in yield due to this pest in cabbage is 31% (Abraham and Padmanabhan 1968). The crop loss is estimated to vary from 52% (Anuradha, 1997) to 100% (Cardleron and Hare, 1986). Timely application of insecticides (Krishnaiah and Jaganmohan, 1977, Krishnaiah *et al.,* 1981) is the only solution to overcome this loss. According to Regupathy (1996), reasons for diamondback moth assuming the status of major pest of crucifers in India are :

i)   Continuous cropping of susceptible crops (cabbage and cauliflower) throughout the year and mono cropping (mustard and rape) in large areas.

ii)   Diversity and abundance of natural enemies (*Cotesia plutellae* Kurdj., *Diadegma semiclausum* Hellen) is reduced by redundant and free use of synthetic non-selective insecticides (monocrotophos, quinalphos *etc*.)

iii)   Greater competitive ability of the pest over its natural enemies in establishing itself in newer areas.

iv)   Ability to migrate longer distances.

v)   Out-dated application technology resulting in inefficient targeting of spray against diamondback moth.

vi)   Short generation period of DBM. As many as 16 generations are completed per year (Jayarathnam, 1977).

3.   Life history

Some work on the bionomics of the DBM has been done in Kodaikanal (Abraham and Padmanabhan, 1968), Jabalpur (Rawat *et al.,* 1968), and in Jobner (Sachan and Srivastava, 1972, Yadav *et al.,* 1974). The biology of this pest has been studied in the laboratory (Patil and Pokharkar 1971, Jayarathnam, 1977) and under natural conditions in relation to ecological factors. The life cycle from egg to adult stage

of the female and the male on an average took 35.65 and 27.95 days, respectively (Bhalla and Dubey, 1986). Yadav *et al.,* (1983) studied the effect of temperature on development of *P. xylostella* and concluded that 25-26°C was the most suitable for development of moth. At Coimbatore, the life cycle lasted for 15-18 days in September and October (Abraham and Padmanabhan, 1968).

3.1. Mating, oviposition and fecundity

The moths mate at dusk on the day of emergence. Mating lasts for one to two hours and females mate only once. Oviposition begins in the evening and lasts upto 7 pm; during rest of the night, moths are not active (Jayarathnam, 1977). Oviposition took place in the evening and during the night. Most females (90%) lay eggs on the same day of emergence. The oviposition period averaged 5.2 days. Patil and Pokharkar (1971) reported that the fecundity ranged from 71 to 203 eggs/female in 1-2 days. Each female laid 220 to 315 (average 284) eggs. The mean fecundity of 153 eggs per female was noticed in Palampur, Himachal Pradesh (Devjani and Singh, 1999), while Sharma *et al.* (1999) reported it to vary from 147-251 eggs. The maximum ovipostion per day by a female ranged from 78-79 eggs. The eggs are generally laid singly or in groups of two to four on the under surface of leaves often along the mid-rib or principal veins (Fig. 1). Typically,
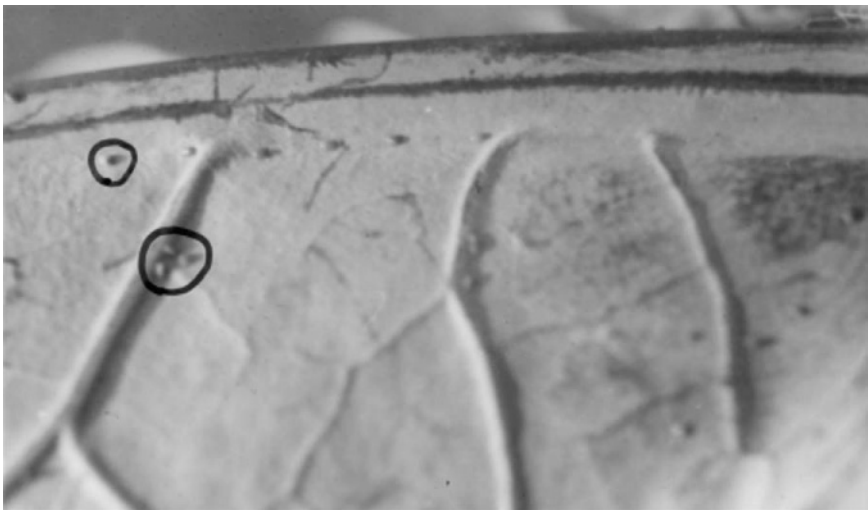
Fig.1. Group of eggs laid under surface of leaf near/on midribs.

eggs are laid in depressions on the leaf along the midrib and larger veins or on concave surfaces near smaller veins.

On an average 63.4 eggs are laid on the upper surface. The eggs are minute, yellowish white, 0.5 mm in size, cylindrical to oblong with an average dimension of 0.48x9.25 mm. The incubation period ranges from 3 to 6 days (Abraham and Padmanabhan, 1968, Patil and Pokharkar, 1971)). Jayarathnam (1977) recorded an incubation period of three to four days under both laboratory and field conditions, while Bhalla and Dubey (1986) reported the mean incubation period of 3.10 days and it was 2.18 days in Manipur (Devjani and Singh, 1999), 3-4 days in Palampur, Himachal Pradesh. The pre oviposition, oviposition and post oviposition periods were 2-4, 6-7 and 5-14 days, respectively (Sharma *et al.,* 1999). The egg viability averaged 95.7%.

3.2. Larva

The freshly hatched larvae are pale white or whitish yellow to pale green with a pale brown head. On an average it measured 1.30 x 0.18 mm. Young larvae after hatching initially wandered over the leaf surface and then fed like miners (Fig. 2). The larvae underwent three moultings resulting in four instars. Total larval period extends from 14 to 21 days (Abraham and Padmanabhan, 1968). Patil and Pokharkar (1971) observed five larval instars in total period of 11 days; however, Jayarathnam (1977), noticed only four instars. The first instar occupies three days in the hot season, 3 to 4 days in the rainy season and 4 to 5 days in the cold season. The larvae generally stay in the mines for about two days. The second instar extends for two days during hot and rainy season and 2 to 3 days in the cold season. The third instar larvae generally feed on mature leaves for two days in the hot and rainy season and for 2 to 3 days in the cold season. The fourth instar larvae, excluding the pre-pupal period, consume the largest quantity of leaf tissue and last for 2 days in the hot season, 2 to 3 days in the rainy and 3 to 4 days in the cold season. Full grown caterpillars are light green, measuring 8.62 to 10.00 mm in length, moderately stout and smooth with short scattered hairs. At the slightest disturbance, the larvae wriggled actively and dropped down the leaf, suspending themselves by silken threads. The total larval and prepupal period are estimated to be 10 days in the hot and rainy seasons, and 12 to 15

Fig.2.   Young larvae wandering on leaf surface

days in the cold season. The pre-pupal period lasts for one day in all
the three seasons. The total larval period averaged 11.3 days (range 9
to 13 days). The duration of four larval instars depends on temperature
(Bhalla and Dubey, 1986). The larval development takes 10.5 days in
Manipur (Devjani and Singh, 1999). It passed through four instars,
the larval periods for the first, second, third and fourth instars being
2-3, 1-1.5, 1-2 and 1.5-2.5 days, respectively at Palampur, Himachal
Pradesh (Bhatia and Verma, 1994).

3.3. Pupa

The mature caterpillar formed a beautiful gauzy, loosely spun cocoon.
Thereafter, it shortened its body longitudinally but remained active.
The newly formed pupa was yellowish green, but in a day or two it
became brownish and gradually attained a dark brown colour by the
time of adult emergence (Fig. 3). Its mean length was 5.15 to 6.00
mm. The average pupal period lasted for 5.85 days. The pupal period
ranges from 7 to 11 days (Abraham and Padmanabhan, 1968), 3 to 7
days with an average of 5 days (Patil and Pokharkar, 1971) and 4
days in the hot and rainy seasons, respectively and 4 to 5 days in the
cold season (Jayarathnam, 1977), 6.86 days in Manipur (Devjani and
Singh, 1995) and 3 to 5 days at Palampur, Himachal Pradesh (Bhatia
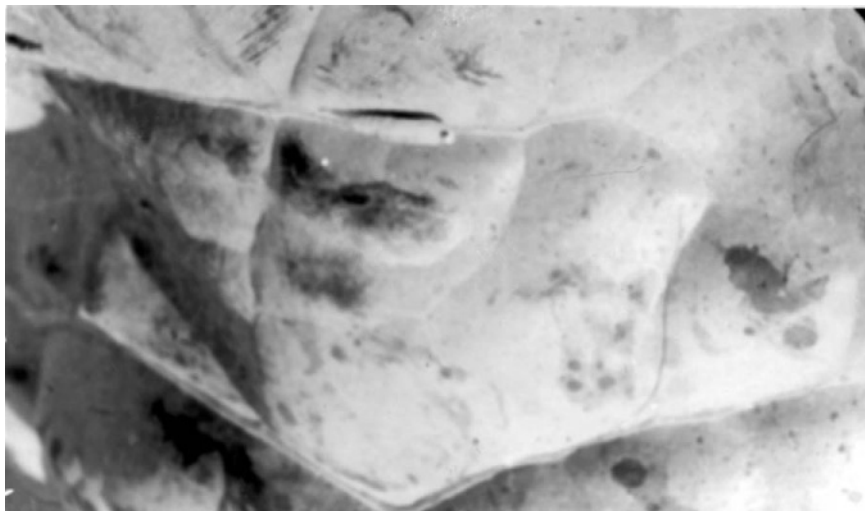
Fig. 3.   DBM Pupa

and Verma, 1994). The duration of the pupal period varies from 4 to 15 days depending on temperature (Abraham and Padmanabhan, 1968; Chelliah and Srinivasan, 1986).

3.4.   Adult and number of generation

Adults are found to emerge in the evening and rarely in the morning hours (Fig. 4). Adult moths are slender and grayish brown with an expanse of 14 mm, and live for 3 to 11 days (Abraham and Padmanabhan, 1968). According to Patil and Pokharkar (1971), life span of male and female is 10 and 12.1 day, respectively, while at Palampur, Himachal Pradesh it was 6-9 and 14 to 20 days, respectively (Bhatia and Verma, 1994). Jayarathnam (1977) noticed the moths to survive for 3 to 6 days without food and for 11 to 16 days with food. The male to female sex ratio worked out to be 1.60:1. Average longevity of the male and the female was 7.7 days. The adult longevity was 16.7 days in Manipur (Devjani and Singh, 1999). It is observed that DBM completes 13 to 14 generations per year in Bangalore (Jayarathnam, 1977). If eggs are laid by the adults of each generation on the same day of emergence, up to 16 generations per year are completed. The respective pre-oviposition, oviposition and post

Fig. 4.   DBM adult

oviposition periods overaged 3.1, 5.2 and 7.4 days. The total developmental period of DBM from egg to adult emergence was 17.8, 20.80, 19.40 and 16.50 days when reared with leaves of Brussels sprouts, cabbages and cauliflowers, knol kohl and sprouting bracoli, respectively under laboratory conditions (Sood *et al.,* 1996).

4.   Host Range

The DBM is an oligophagous pest, known to feed on plants that contain mustard oils and their glycosides. In India, it infests important crucifers *viz*., cabbage (*Brassica oleracea* var. *capitata* L.), cauliflower (*Brassica oleracea* var. *botrytis* L.), radish (*Raphanus sativus* L*.),* beetroot (*Beta vulgaris* L.), knolkhol/kholrabi (*Brassica oleracea* var. *gongyloidis* L.), mustard (*Brassica juncea* L.) and rapeseed (*Brassica napus* L.) (Chand and Choudhary,1977; Dubey and Chand, 1977; Jayarathnam, 1977; Singh and Singh, 1982). Non-cruciferous crops, like *Amaranthus viridis* L. has also been reported to be the host of this species (Vishakantaiah and Vishweshwaragowda, 1975). In Northwestern Himalayan region except cabbage and cauliflower no other popular cruciferous crops (turnip, radish, knolkhol, kale and mustard) were found to be infested by this pest (Bhalla and Dubey, 1986).
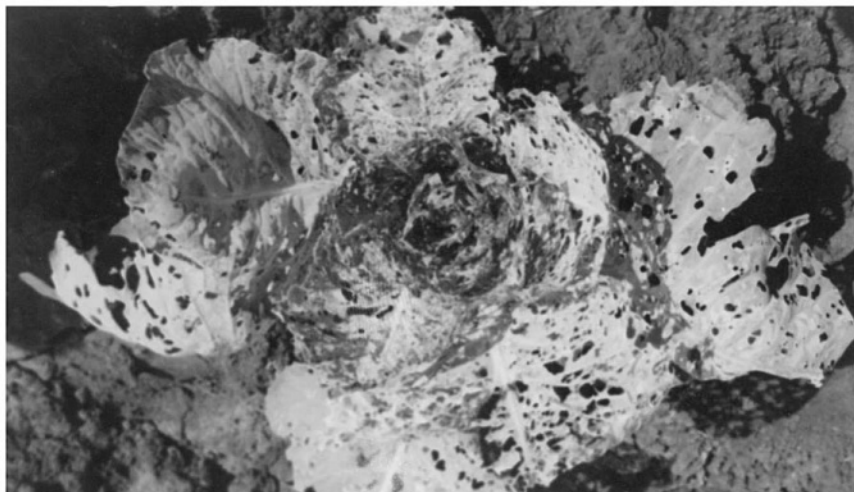
Fig. 5.   DBM infestation on cabbage

The pest exhibits a marked preference for cauliflower and cabbage (Fig. 5) as they posses fleshy and succulent leaves that probably provides olfactory and gustatory stimuli for successful host selection and development (Chand and Choudhary, 1977; Dubey and Chand,1977; Singh and Singh, 1982).

5.   Seasonal incidence

Peak activity of DBM larvae in cauliflower fields was observed during December - January, when there was considerable fall in the minimum temperature which fluctuated between 0 to 1.0°C while the maximum temperature ranged from 21° to 24°C (Lall, 1939).

Morgan (1929), Prasad (1963) and Sachan and Srivastava (1972) reported that infestation of plants varied from 5% to 100% during their experimental crop growth seasons. Similarly Bhalla and Dubey (1986) observed the incidence of attack in North western Himalaya. Between 3% and 73% plants were infested with larval population of between 3 and 41.5 per 100 plants.

Damage of diamondback moth is reported to be severe from July to September. Seasonal incidence of DBM on cabbage has been studied in India at Kodaikanal (Abraham and Padmanabhan 1968)and high build up of larval population has been reported during February-March (late winter) and April-August (Summer and mid rainy season).

Infestation by larvae and pupae of *P. xylostella* averaged 2-24 per plant at different months in the year, 7-8% of the plants being infested in September-October, when infestation started, and 32-100% in January-March at the peak in Jobner, Rajasthan (Sachan and Srivastava, 1972). A small populations of caterpillars of DBM was noticed in winter at Bangalore, while Verma *et al*, (1972) observed a serious infestation in cauliflower fields around Hissar during August.

Seasonal incidence of DBM on cabbage has been studied in India at Bangalore (Jayarathnam 1977). He found significantly high build up of larval populations during the rainy season (July-September) as compared to other seasons. He also studied the population dynamics of the pest by preparing life tables for 10 generations (five in rainy season and five in cold season). Major mortality causes have been parasitization by *Apanteles plutellae* Linnaeus in the larval period, predation by predatory ants, birds and spiders in the 1st and 2nd larval instars, and parasitization in pupal stage by *Tetrastichus sokolowskii* Kurdj in all the 10 generations. Infestation by the cabbage webworm *Hellula undalis* Zell. has been found causing considerable reduction of oviposition sites for DBM. DBM was observed throughout the year except during the winter season in Karnataka. The larvae are noticed 28 days after transplanting till the harvest of the crop; while Dhaliwal and Goma (1979) from Solan reported the peak period of activity of *P. xylostella* on cole crops between April and August. Nagarkatti and Jayanth (1982) studied the seasonal incidence of DBM on cabbage in Bangalore district and found significantly high buildup of larval population during the rainy season (July-September) as compared to other seasons.

In the North western Himalaya, the pest seriously damages the cabbage crop during September and October in dry cold areas but was seen only in small numbers on off season cabbage and cauliflower crops grown during wet months (June-September) and on the cauliflower seed crop in December-May (Bhalla and Dubey 1986). The effect of planting date of cabbage on the level of damage by *P. xylostella* indicates that highest percentage of leaf damage of 98.83% was recorded in the crop planted in the 1st week of January followed by that planted in the 1st week of December (16.87%); however, highest average yield per plot (12.2 kg) was recorded in the crop planted in the 1st week of October (Talekar and Griggs, 1986).

The peak incidence of the pests in Maharashtra on cabbage was noticed in first fortnight of February and the maximum yield was obtained when transplanting was done during November-December (Khaire *et al.,* 1987). Higher incidence of the pest during kharif than in winter-summer on younger plants supporting more larvae as compared to older plants has been observed in Northern Karnataka. The incidence of *P. xylostella* on winter crop of cabbage was noticed by the end of January and peak population was observed between last week of March to third week of April in Himachal Pradesh as reported by Bhatia and Verma (1994). Later the incidence of DBM on summer crop of cabbage was reported in the month of June and peak larval counts at the end of July-August in Himachal Pradesh (Bhatia and Varma, 1995).

The population of DBM in Andhra Pradesh reached maximum by the last week of February and declined there after. Distribution pattern of larvae in the field is aggregative in nature. This provides clumping behaviour of individuals in a population (Narendra Reddy *et al.,* 1996). Sujatha *et al., (*1997) from Andhra Pradesh opined that December and January months favoured multiplication of *P. xylostella* and peak population was noticed in the month of January. Usha *et al*. (1997) reported the peak larval population of *P. xylostella* in the first week of April. Similarly, Devjani and Singh (1999) observed maximum field density of *P. xylostella* during March 1994 and 1995 cropping season in Manipur.

In the terrain region of West Bengal, spring cabbage was infested by greater pest populations of DBM compared to winter cabbage. Increase in temperature, sunshine hours per day and rainfall and decrease in relative humidity favoured the multiplication of the pest on spring cabbage (Chaudhari *et al.,* 2001).

6.   Economic threshold

Seven weeks after transplanting cabbage could sustain populations of 20 larvae/plant before significant economic injury and yield reduction were detected (Prasad, 1963). Based on crop loss estimation studies conducted at Bangalore, Jayarathnam (1977) found that a population of four or more medium sized larvae (3rd or 4th instar) could render a

seedling un-transplantable and ten medium sized larvae per plant up to one month between one and two months after planting necessitated insecticide application. Early instar larvae make small cavities and holes in the leaves and seldom mine into the older leaves. Later instar caterpillar extensively feed upon the leaf tissues from the lower surface leaving only the upper epidermis and veins. Even the heads are bored by the caterpillars (Krishnaiah *et al.,* 1981). Path coefficient analysis indicates that DBM infestation at 55 days after planting has maximum negative effect in reducing the yield (Chelliah and Srinivason, 1986). The larval population at 20, 30, 40, 50 and 60 days after planting and the marketable yield showed significant negative relationship for insect count at 40,50 and 60 days after planting (Srinivasan 1984). Multiple linear regression equation for these larval population in relation to loss of marketable yields is: $Y = -168.79\, X_1 + 84.33\, X_2 - 98.43\, X_3 - 21.9\, X_4 - 115.92\, X_5$ where x1, x2, x3, x4 and x5 correspond to population on 20, 30, 40, 50 and 60 days after planting (Srinivasan, 1984). Srinivasan and Veeresh (1986a) developed visual damage thresholds for the chemical control of *P. xylostella* on cabbage. One standard hole of size 0.5 to 1.00 cm in diameter was taken as the economic threshold for the chemical control.

Action threshold for initiating insecticide spray against *P. xylostella* based on percentage damage to the head was evaluated in comparison with calendar based weekly and fortnightly sprays. There was no significant difference in the marketable yield between plots maintained with thresholds of 3 and 5% new damage and those with fixed spray programmes (Srinivasan *et al*., 1988). Damage by the larvae before cupping did not contribute to economic yield loss. Defoliation of wrapper leaves significantly reduced the marketable yield. Larval population recorded on 40 days after planting (DAP) and escalating thereafter had significantly negative correlation to marketable yield. Insecticide protection from 7 to 10 days before this date (40 DAP) could prevent the pest population reaching economic injury status. Multiple correlation and linear regression revealed that 90 to 92% yield loss could occur if cabbage was left unprotected (Srinivasan, 1984). Consequent to the maximum negative effect of DBM population that occurred on 60 DAP on yield, it was concluded to have a blanket insecticide spray at this stage. Based on visual damage

threshold, the plots maintained with 0.5 to 2.0 mean holes in wrapper leaves required four spray applications until harvest. Yield obtained in these plots were at par with yield recorded in those sprayed with 0.07% phosalone nine times at weekly intervals. A maximum additional net income of $ 606/ha over control was obtained in the plots maintained with damage threshold of 0.5 holes in wrapper leaves (Srinivasan, 1984). Reddy and Guerrero (2001) reported the optimum timing of insecticide applications against DBM in Cole crops using threshold catches of 8, 12 and 16 male moths/trap/night in cabbage cauliflower and knol khol crops respectively and this was more effective than regular sprays.

7.   Natural enemies

The natural enemies recorded on DBM in India by various authors are summarized in Table 1.

TABLE 1:
Natural enemies of diamondback moth (*Plutella xylostella*)

Sl. No.	Species	Stage	Remarks	References
	I. Parasitoids			
1.	*Trichogramma chilonis* Ishii (Trichogrammatidae: Hymenoptera)	Egg	51.5-57.0% parasitization when released @ 2 lakhs/ha	Anuradha, 1997
2.	*Trichogramma armigera* Nag (Trichogrammatidae: Hymenoptera)	Egg	-	Manjunath, 1972
3.	*Trichogrammatoidea bactrae* Nagaraja (Trichogrammatidae: Hymenoptera)	Egg	30% parasitization	Singh and Jalali, 1993
4.	*Cotesia (Apanteles) plutellae* Linnaeus (Braconidae: Hymenoptera)	Larva	18-75% parasitization. Peak activity - July and October	Nagarakatti and Jayanth, 1982

Tables contd.

	-do-		72% parasitization	Yadav *et al.* 1975
	-do-		80% parasitization	Jayarathnam 1977 & Chandramohan, 1994
	-do-		More Prevalent in rainy season than in cold	
5.	*Diadegma fenestrale* Holmgren *Diadegma collaris* Gravenhorst	Pupa	66 to 80% parasitization	Chauhan *et al.* 1997 and Devi and Raj 1995
6.	*Diadegma semiclausum* Horstmann	Pupa	68% parasitization	Chandramohan, 1994
7.	*Tetrastichus sokolowskii* Kurdj (Eulopidae:Hymenoptera)	Larval-pupal	Endoparasitoid, Parasitization – 30 to 78% Peak activity-August-September	Nagarakatti and Jayanth, 1982
8.	*Brachymeria exacarinata* Gahan (Chalcididae:Hymenoptera)	Pupa	Endoparasitoid, parasization-20 to 60% peak activy-August	Cherian, 1938
II. Predators				
1.	*Chrysoperla carnea* Stephens (Chysopidae:Neuroptera)	Egg & Larva	Single larva predate 74.67 eggs and 57.0 first instar larvae	Anuradha, 1997
2.	*Coranus* sp. Reduvidae : Hemiptera	Larva	Single adult predate 12 DBM larvae/day. Optimum predator prey ratio 1:6	Anuradha, 1997
Ants				
1.	*Tapinoma melanocephalum* Formicidae:Hymenoptera	Larva	Sprinkling 5% jaggery solution encourages ant activity	Jayarathnam, 1977
2.	*Camponotus sericus* Formicidae:Hymenoptera	Larva	-do-	"
3.	*Pheidole sp* Formicidae:Hymenoptera	Larva	-do-	
Birds				
1.	Yellow wag tail (*Motacilla flava*)	Larva	Peak activity during cold seasons	Jayarathnam, 1977
2.	Cattle egret (*Bulbueus ibis*)	Larva	-do-	"
III. Pathogens				
1.	*Bacillus thuringiensis* var *kurstaki*	Larva	-	Narayanan *et al.*1970

Cont. on next page

Tables 1 contd.

2.	Nuclear polyhedrosis virus (NPV)	Larva	-	Anuradha, 1997
3.	Granulosis virus (GV)	Larva	-NA-	Rabindra *et al.*1996
4.	*Paecilomyces farinosus* (Fungus)	Larva	Spraying fungal inoculum @ 1.7 x 10^8 spores/ml at weekly interval from initiation of primordia	Anuradha, 1997
5.	*Beaveria bassiana* (Fungus)	Larva	Spray of conidial	Voon *et al.* 1999
6.	*Zoophthora radicans* Fre (fungus)	Larva	-	Gopal Krishnan,1998
7.	*Vairiomorpha* sp. (protozoa)	Larva	Spraying inoculum @1.2 x 10^7 spores/ml reduce DBM population by 55.7%	Anuradha, 1997
8.	Nematode (DD-136)	Larva	Reduce DBM population by 15.5%	Anuradha, 1997

8. Management tools

8.1. Cultural practices

8.1.1. Intercropping / Trap cropping

There was no reduction of DBM when tomato and cabbage planted at the same time. However, planting of tomato a month earlier to cabbage in 1:1 row caused greater reduction of DBM larvae on cabbage. This is attributed to the release of substances from and covering of cabbage leaves by the spreading foliage of full-grown tomato plants (Srinivasan, 1984). However, inter-cropped cabbage did not show any significant increase in the marketable yield of cabbage compared to that from sprayed plots (Srinivasan and Krishnakumar, 1982). Significant reduction in number of DBM larvae when cabbage was planted 30 days after the tomatoes and to a lesser extent when they were planted 15 days after the tomatoes, in comparison to when cabbages were planted alone (Srinivasan and Veeresh,1986b).

Indian mustard has been found to be a preferred host for oviposition by *P. xylostella* than cabbage in the laboratory studies. Field trails in Bangalore also confirmed that DBM prefers mustard over cabbage when the choice is available. Therefore, the ideal inter-crop combination suggested is nine rows of cabbage followed by 1 paired row of mustard. In one of these rows, mustard is sown 15 days before and in the other row, 25 days after planting cabbage. Further studies have indicated that planting pattern of 15 rows of cabbage followed by mustard rows is most promising for the successful management of the pest. Mustard is

sprayed with 0.1% dichlorvos at 10 days interval commencing from 12 days after sowing to suppress the insect pests. Cabbage can successfully be raised by inter-cropping strategy during the rainy season without insecticidal application, while two sprays with 0.05% cartap hydrochloride are necessary during winter (Srinivasan *et al.,* 1991, Srinivasan and Moorthy, 1991).

8.1.2. Resistant varieties

Early maturing cauliflower varieties were tested in the farmers field near Varanasi for their reactions to *P. xylostella* infestation. Based on DBM infestation index and its mean relative yield, varieties Pusa ketki and Pusa deepali were found to be highly resistant and early Kunwari, Pant Gobhi-3, Early Pusa synthetic, Sel-327, Pusa hybrid-2 and Pant subbra as moderately resistant. The least resistant reaction to DBM was observed in case of Pusa sharad and Sel-328 (Mathuram and Raju, 2002).

8.2. Botanicals

Botanicals *viz*., derris root and seeds of *Tephrosia candida (*Roxb.) DC and *Annona squamosa* L. are effective against *P. xylostella*. Treatment with hot industrial alcohol extracts containing active principles from *T.candida* and *A. squamosa* seeds at 1.00% has been toxic to *P. xylostella*. It is considered that these materials and the seeds of *A. squamosa* are likely to be of commercial value as insecticide (Puttarudraiah and Bhatta, 1955). The studies from AVRDC, Taiwan indicated the influence of tomato leaf extract (200 g in 2l) on oviposition of DBM in treated common cabbage and Chinese cabbage plants. The results indicated that, significant reduction in number of eggs laid by DBM on treated plants.

Methanolic extracts of seeds of *Thevetia nerifolia* Merr, *Pongamia pinnata* L. and roots of *Nerium oleander* L., all at 1.0% resulted in 100% mortality of fourth instar larvae of *P. xylostella,* 12-24 h after treatment when applied topically. The plant extracts viz., *Lobelia excelsa, Lantana camara* L*, Pteridium aquilinum, Helichrysum buddleoides* and *Acorus calamus* L. applied at 500 ml/ ha with a high volume sprayer are more effective than monocrotophos at 0.05% (Satpathi and Ghatak, 1990). Chandramohan and Nanjan (1990) showed that a neem oil product called 'Biosol' (0.4% active)` was 3 to 4 times more effective in controlling *P. xylostella* compared to control and organic garlic spray. However, contrary to this, the

insecticide shows higher yield than *A. calamus* extract (Rajavel and Veeraraghavathatham, 1989). When cabbage leaves treated with acetone extract of xerophytic perennial plant, *Agave cantala* Roxb., the extract was found to be weakly toxic at the highest concentration of 60 mg per ml, but it prolonged the larval and pupal periods, decreased the percentage of adult emergence and adult longevity (Reddy and Urs, 1991).

Studies were carried out at Anand, Gujarat by Patel *et al*. (1993) to evaluate locally available plant materials for anti-feedant / insecticidal properties against the larvae of DBM. Neem seed kernel paste (5% suspension) was effective against the pest. Repelin was best whereas, neemark 1.0% was at par with neem seed kernel paste against *P. xylostella.* Srinivasan and Moorthy (1993) reported that 4 and 5% aqueous extract of neem seed kernal consistently recorded significant reduction in DBM larvae with consequent increase in table heads compared to cartap hydrochloride (.01%), endosulfan (0.07%) and one% neemark and repelin. Raju *et al*. (1994) compared the efficacy of an unspecified neem oil extract and a range of commercial synthetic pesticides on two different parent strains of *P. xylostella*. These workers recorded low% mortality with neem oil extract treatment in both the strains.

Up to 71% reduction in egg lying on glass jars treated with methanolic extract of *M. azedarach* than those treated with solvents only, (Dilawari *et al.,* 1994). Methanol extracts of *M. azedarach* reduced feeding in second instar larvae. They also reported that diethyl ether fraction was most potent in larvicidal and anti-feedant action. A laboratory experiment was conducted in Tamil Nadu by Justin *et al*.(1995) with plant extracts (NSKE at 5%, *Catharanthus roseus* L. and *Ocimum sanctum* L. at 3%) either alone or in combination with *B. thuringiensis* against third instar larvae of DBM. NSKE and *C. roseus* showed antifeedant effect with reduction in cauliflower leaf consumption. Sannaveerappanavar (1995) made claims of potency of NSKE at 0.5, 1.0, 2.0, 3.0 and 4.0% concentration on the development of second, third and fourth instar DBM larvae. The NSKE at all the concentrations tested was highly detrimental to the second instar larvae causing cent% mortality. Even on third and fourth instar larvae, NSKE at 2.0, 3.0 and 4.0% caused almost complete mortality;

while, at lower levels (1 and 0.5%), lesser level of larval mortality was observed.

The bioefficacy of four indigenous plant materials and insecticides against the major insect pests of cabbage was estimated for two years during 1993-94 and 1994-95. (Singh and Khuman, 1997). Monocrotophos at 0.03% was found to be the most effective against all the pests, however indigenous plant material *Jatropha* sp. at 0.1% was found to be at par and equi-effective with chemical insecticides. Studies of Sannaveerappanavar *et al*. (1997) suggests that NSKE 4.0% can be effectively used in managing the resistant population of DBM. *Andrographolis paniculata* a main constituent of tropical shrub has found to have oviposition deterrent activity against the diamond back moth. The choice experiment showed that 250, 500 and 1000 ppm andrographolide spray reduced moth oviposition by 72 to 81 (Hermawan *et al*. 1998). Studies carried out in Tamil Nadu, showed that three applications of neem seed kernel extract were adequate to reduce the population of *P. xylostella* (Moorthy *et al*., 1998)

Four applications of *Azadirachta indica* (7.50%) was as effective as new chemicals (Profenofos 50 EC, Profenofos 40% + Cypermethrin 4%) against *P. xylostella* in cabbage by recording cent% reduction in larval population after 3 days. *A. calamus* (7.5%) and Honge oil (0.4%) also proved effective in reducing the DBM population to the extent of 88.60 and 90.64% respectively over control.

8.3. Insect growth regulators (IGRs)

Diflubenzuron posses strong anti-feedant properties against second and third instar larvae (Suresh and Sundarababu, 1989), but teflubenzuron (45 g.a.i./ha) and flufenoxuron (20 g.a.i./ha) are more effective (Peter and Sundararajan, 1991). Teflubenzuron 90 g.a.i./ha is highly promising in reducing larval population and it remained at par with 60 and 150 g.a.i./ha (Jadhav *et al.,* 1992a). Jadhav *et al*. (1992 b) and Somchaudhary *et al*. (1996) recorded the similar trend on bio efficacy of flufenoxuron against DBM on cabbage at dosages ranging from 30-40 g.a.i./ha . Kadam *et al*. (1995) opined that the% un-hatched eggs of DBM ranged from 22.4 to 100 and 17.5 to 77.5 at various concentrations of difleubenzuron tested for newly oviposited

eggs and eggs prior to hatching respectively, compared to 10 and 3.33 for untreated control. In a field trial lufenuron at 600 or 400 ml per ha was found to be highly toxic to DBM based on the% reduction in larval population in cauliflower (Ranganathan and Govindan, 1996). Similar trend was noticed by Verma (1997) with lufenuron at 0.006%, Sannaveerappanavar *et al.* (1997) at 20, 30 and 40 g ai./ha in cabbage and Sangareddy *et al*.(1999) using novaluron at 0.50%, 0.05% and 0.1% in cauliflower.

Two applications of teflubenzuron (90 g.a.i./ha) mixed with urea (2%) significantly reduced the larval population from 4.08 to 0.24 larvae/head and recorded highest (17.53 t/ha) cabbage yield (Krishna Naik *et al.,*1997). This is due to synergisation of IGR to inhibit chitin synthesis and feeding in DBM larvae. Three sprays of flufenoxuron (80 4.a.i./ha) reduced the infestation at 3, 7 and 10 days after application significantly more than fenvalerate 40 g.a.i./ha. Delfin (Bt) 50 WG 0.5 kg/ha and cartap hydrochloride (400 g.a.i./ha) reduced the infestation of DBM and increased the cabbage yield (Tambe *et al.,* 1997). Lufenuron at 20 g.a.i./ha reduces the pest population and significantly increases in marketable heads (Sannaveerappanavar *et al*., 1997). Maheshkumar *et al*. (1999) concluded that novaluron at 0.1 and 0.75% was found to be best against DBM in cabbage. Anureet Josan *et al.* (2000) from Punjab reported sub-lethal effects of a novel insect growth regulator in larvae, pupa and adults of *P. xylostella.*

8.4.   Microbial pesticide

8.4.1. *Bt* and *Bt* - based formulations
Larvae of *P.xylostella* suffer with 100% mortality both in laboratory as well as greenhouse when *Bt* is applied in the form of dust, wettable powder and emulsion. Even in field, complete control of the pest is achieved three days after application of the pathogen (Narayanan *et al*., 1970). They tested the effectiveness of four commercial preparations of *B. thuringiensis* along with NPV specific to DBM under lab condition. They found thuricide HPSC and dipel WP to be more promising at 1.0 and 1.5% product per litre of water than bactospeen or thuricide 90 TS. Rajamohan and Jayaraj (1978) reported the persistence of *Bt* for about 10 days. Weekly spray with dipel (0.5 kg product/ha) is quite effective and comparable to that of fortnightly spray of methamidaphos and quinalphos (0.5 kg.a.i./ha) (Krishnaiah, *et al*.,1981).

Field trails conducted in South India on the control of the *P. xylostella* on cauliflower, *B. thuringiensis* has given better control of the pest and higher yield than all the chemicals insecticides tested (Justin *et al.,* 1990).

Four field trials were conducted; two each with cabbage and cauliflower to assess the bio-efficacy of carbosulfan (Posse 25 EC), biobit (*B. thuringiensis* var *kurstaki* based formulation), neem oil 50 EC against DBM in comparison with commonly used insecticides, cartap and quinalphos. The results indicated that cartap (250 g.a.i./ha), carbosulfan 9250 g.a.i./ha) and biobit (750 g) were found to be highly effective in lowering larval population and increasing yield. Neem oil 50 EC did not affect perceptible reduction in DBM population (Chandrasekaran *et al*., 1994).

Several *Bt* formulations were tested in different parts of the country and found effective in reducing larval population and increasing the yield substantially (Justin *et al.,* 1990; Asokan and Mohan, 1996, 1999; Sharma *et al.,* 2001). Of the eight subspecies of *B. thuringiensis* var. *kurstaki* (Bactospeine) at 10^7/ml spores is most toxic to the third instar larvae of *P. xylostella* followed by *B. thuringiensis* in the laboratory bioassay tests (Justin, 1996). *Bt* formulations - Delfin, Dipel, Halt and Biobit were found superior in the control of DBM.

Significantly higher marketable cabbage heads are obtained with delfin (*B. thuringiensis*) at 0.5 and 10 kg/ha (Panchabhavi and Sudindra, 1994; Kulkarni *et al*. 1995 and Sannaveerappanavar, 1995). While Justin *et al.*, 1990 did not notice improvement in the efficacy of bacterial pathogen when combined with endosulphan, fenvalerate and diflubenzuron. Justin *et al.* (1999) reported that yeast extract followed by egg albumin as very good UV protectants for *B. thuringiensis* against DBM on cauliflower.

Several authors have reported the efficacy of *Bt* against DBM. Kulkarni *et al.* (1995) with deflin at 0.5 kg and Wock biological 01 at 1 kg/ha, Asokan *et al.* (1996) with *Bt* sub species *kurstaki* at 1 ml/l and *Bt* subspecies *aizawai* at 1 g/l and Nagesh and Verma (1997) with Biolep (0.2%). In a field trial thuricide + triazophos (0.08%+ 0.06%) combination treatment was found to be the most beneficial against DBM followed by thuricide alone (0.08%) in cabbage. The new formulation of *B. thuringiensis* sub. sp. *kurstaki* was effective against DBM at 0.50-1.0 g/l giving 92.1% and 100% mortality against 1st and 3rd instar larvae, respectively. *Btk* at 1.0 g/l also gave cent% mortality against third instar larvae. The formulation was safe to *Trichogrammatoidea bactrae* (Singh *et al.,* 2000). Loganathan *et al.* (2001) reported that Spicturin *(B. thuringiensis var galleriae)* can be combined with *C. plutellae* for the management of DBM as it was safe to the parasitoid.

8.4.2 Fungal, viral and nematode pathogens

The entomopathogenic fungi, *Pacecilomyces farinosus* (Holmskiold) Brown and Smith and *Zoophthora radicans* (Brefeld) Batko were isolated from *P. xylostella*. *P. farinosus* was tested for its field efficacy against DBM on cabbage. Application of five rounds of the fungus @ 1.7 x 10^8 spores/ml + Triton X- 100 (0.01%) at weekly intervals significantly brought down the larval population of DBM and increased the marketable yield (Gopalakrishnan, 1998). Kennedy *et al*. (2000) also indicated the scope for utilizing the fungus *Beauveria bassiana* (Bals.) Vuill. in the management of DBM.

A granulosis virus has been isolated from *P. xylostella* By PDBC, Bangalore and TNAU, Coimbatore. The potential of these pathogens has to be exploited for the management of DBM in Cole crops.

The percent reduction of DBM by the entomopathogenic nematode *Steinemema carpocapsac* was observed tothe extent of 10-30% over the dosage tested (Ratnasinghe and Hague, 1998).

Yan, *et al*. (1999) Testing *Steinernema carpocapsac* A-24 and *Heterorhabditis bacteriophora* 8406 as the best strains of entomopathogenic nematodes for *P. xylostella* control under field conditions in China. the mortality rate was observed to the extent of 33.9% in comparison with the use of a humidifier.

Eight entomopathogenic nematodes were tested against the final instar larvae of DBM. *Heterorhabditis bacteriophora* was most pathogenic amongst the test nematodes on the basis of LD_{50} (9.16 IJS/larva) LT_{50} (43.26 hrs), Lex T_{50} (3.24 hr) and the propagation potential (average of 271.42 IJS / mg) on the host body weight (Shinde *et al.,* 2000).

8.4.3. *Bt* transgenics

A synthetic *Bacillus thuringiensis (Bt)* cry 1 C gene was introduced into broccoli by *Agrobucterium tumefacions* mediated transformation. This study demonstrated that high production of cry 1 C profane can prefect transgenic broccoli not only from susceptible or cry 1 AR DBM larvae but also from DBM selected for moderation levels of resistance to cry 1 C (Cao *et al.,* 1999).

A synthetic Cry 1A(b) gene coding for an insecticidal crystal protein of *B. thuringiensis* (*Bt*) was transferred to cabbage cultivar Golden Acre by co-cultivating hypocotyl ex-plants with *Agrobacterium tumefaciens*. Transformed plants resistant to kanamycin were regenerated. Hybridization experiments demonstrated gene integration and mRNA expression. Immunoblot analysis revealed high-level expression of *Bt* toxin protein in the transgenic plants. The expression resulted in significant insecticidal activity of transgenic cabbage plants against the larvae of DBM. The results also demonstrated that a synthetic gene based on monocot codon usage can be expressed in dicotyledonous plants for insect control (Bhattacharya *et al.,* 2002).

8.5.   Chemical control

Successful cultivation of cabbage is hampered due to the incidence of DBM on Cabbage in Bangalore (Nagarakatti and Jayanth, 1982). Even though economic threshold for the pest is on record, it is not adopted by growers due to lack of training, hard work and skill in the diagnosis of the pest. However, use of threshold based on quick visual ratings (as revealed by the appearance of holes in leaves caused by feeding of caterpillars) requires little formal training and time. After the application of blanket spray to protect the primordia/head formation

stage, further sprayings are restricted to the number necessary to keep damage to not more than one hole on an average per wrapper leaf of the cabbage. This approach reduces the number of pesticidal applications by 3 to 5 as against weekly spraying (Srinivasan, 1984). It is also possible to eliminate pre-heading sprays, since the larval population causing damage to either outer leaves, or to leaves about to cover the head does not reduce marketable yield significantly. Srinivasan and Veeresh (1986a) developed visual damage thresholds for the chemical control of *P. xylostella* in cabbage under Indian conditions. One standard hole of size 0.5 - 1.0 cm in diameter was taken as the economic threshold for the chemical control.

8.5.1. Use of insecticides

Application of carbofuran (furadan 3 G) at 1.5 kg a.i./ha at the time of transplanting of the crop was observed to be most effective in suppressing the DBM population and in increasing yield (Abraham and Padmanabhan, 1968). The highest yield was recorded when cabbage was treated with cartap hydrochloride (100 g.a.i./ha) followed by fenvalerate (75 g.a.i./ha) (Chelliah and Srinivasan, 1986, Mohan, 1987, Peter *et al.*, 1989 and Rajavel and Veeraragavathatham, 1989). Excellent control of DBM has been reported by many workers (Gupta and Sharma, 1971, Joshi and Sharma, 1976, Gowds *et al.,* 1977, Krishnaiah and Jagan Mohan, 1977, Rajmohan and Jayaraj, 1978, Ramasubbaiah and Lal, 1978, Sachan and Srivastava, 1975, Sarode and Kumar, 1983 and Srinivasan and Krishnakumar, 1982) by using dimethoate, endosulfan, phosphamidon, malathion, quinalphos, chlorpyriphos, phosalone, phenthoate, methomyl, permethrin, lufenuron, acetamiprid and fenvalerate. Evaluation of insecticides on cauliflower, grown as a seed crop, has shown that sprays of phosalone at 0.05% or phenthoate at 0.5% are effective against DBM larvae (Regupathy and Paranjothi, 1980).

The residue of phosalone sprays at 0.05% and 0.1% reaches below tolerance limit of 1 ppm in 5.91 to 8.94 days, and the half life values are reported to be 3.1 and 2.83 days, respectively. The effectiveness of synthetic pyrethroids *viz.,* cypermethrin at 60 and 80 g.a.i./ha, fenvalerate at 80 and 100 g.a.i./ha, permethrin at 125 g.a.i./ha and deltamethrin at 75 g.a.i./ha for the control of larvae has been reported (Gandhali *et al*., 1982). Treatment with methamidophos (0.05%) was found to be most effective for the control of diamondback month (Gandhali *et al*., 1982).

The calendar-based system of four sprays was found preferable to reduce the pesticide load in the environment. It was found that the system using six sprays treatment was approximately the same to that of four sprays in terms of net returns (Rs. 29,400 - 30,800 / ha). (Mallapur *et al,* 1994).

The lowest larval population of *P.xylostella* was recorded following treatment with deltamethrin (20ppm), cypermethrin (100ppm) and lambda-cyhalothrin (62.5 ppm). However, lowest infestation and highest yield was recorded

with lambda-cyhalothrin 62.5 and 50 ppm as compared to cypermethrin 0.001%, deltamethrin 0.002%, monocrotophos 0.05% and endosulfan 0.07% (Rajavel *et al.,* 1991, Rajavel and Gopalan, 1991). Diafenthiuron 50 SC (0.12%) was superior to quinalphos 25 EC (0.05%) and neemark (0.4%). chlorpyriphos (0.05%) or Phosalone (0.05%) when applied to the just opened floral buds of cauliflower seed crop gave best control of *P. xylostella* seven days after treatment (Gupta *et al.*, 1985). Three spays of spark 36 EC (triazophos 35%+deltamethrin 1%) @ 2500 ml/ha at an interval of 10 days starting from 40 days after transplanting recorded highest yield (27.41 t/ha) of cabbage heads (Walunj *et al.,* 1997). From Haryana (Kalra and Sharma, 2000) it was reported that thiodicarb at 1.25 kg/ha causing highest mortality of *P. xylostella* infesting cauliflower. Peter *et al. (*2000) from Andhra Pradesh reported the bioefficacy of Spinosad against *P. xylostella.* Quinalphos followed by Endosulfan, Biobit, Biolep, Cypermethrin and Fenvalerate treatments were found to be most effective in controlling the DBM (Nathuram *et al.*, 2001).

8.5.2. Use of selective insecticides

Studies on relative toxicity of certain conventional insecticides and synthetic pyrethroids have been made against the adults and cocoons of *A. plutellae.* The results indicate that permethrin, fenvalerate, cypermethrin, deltamethrin and phosalone were safer to adults and cocoons of *A. plutellae;* quinalphos was found to be detrimental to both stages of this parasitoid. Dichlorvos, monocrotophos and endosulfan were found to be highly toxic to adults but relatively safe to cocoons of *A. plutellae* (Mani and Moorthy, 1984).

        Neem Seed Kernel Extract was found to be safest to *C. plutellae* followed by neemark and acephate; while, cartap hydrochloride, quinalphos, methomyl, endosulfan, monocrotophos, fipronil and fenvalerate were highly toxic to both adults as well as the immature stages of *C. plutellae.*

        Six insecticides *viz*., fluvalinate (0.01%), carbaryl (0.01%), acephate (0.1%) methyl demeton (0.05%), NSKE (2%) and neemark (2%) were completely safe to *C. plutellae;* while, chlorpyriphos (0.05%) and methyl parathion (0.05%) had quick knockdown effect causing 100% mortality within one hour of exposure (Mani, 1995). Most of the conventional insecticides have killed the key parasitoids of the pest resulting in the increased populations of DBM in recent years. On the other hand, non-conventional insecticides like neem seed kernel extract and the microbial pathogens (*Bt* and fungus) help in managing the pest population without reducing the populations of local natural enemies.

8.5.3. Insecticide resistance and management

The insecticide resistance in diamondback moth is wide spread and covers all major groups of insecticides (Table 2). DBM was not reported as major pest of crucifers prior to the introduction of synthetic insecticides in late 1940's. However, with widespread use of synthetic insecticides on crucifers in the mid 1950's, important natural enemies got eliminated (Talekar and Shelton, 1993); while, continued usage of synthetic insecticides resulted in the development of insecticide

TABLE 2 :

Reported cases of insecticide resistance in DBM in India

Insecticide	Resistanmce level (Folds)/ 5 survival		Reference
ORGANOCHLORINES			
DDT	ND	India	Verma and Sandhu (1968)
		India	Deshmukh and Saramma (1973)
		India	Chawla and Kalra (1976)
HCH / -BHC	ND	India	Deshmukh and Saramma (1973)
		India	Chawla and Kalra (1976)
Endosulfan	Moderate	United States	Magaro and Edelson (1990)
	25%	United States	Yu and Nguyen (1992)
	High	Mexico	Diaz *et al*. (1994)
	16.26	India	Sannaveerappanavar (1995)
	5.00	India	Raju and Singh (1995)
	61.23-79.96%	India	Vastrad (2000)
Endrin	ND	India	Deshmukh and Saramma (1973)
		India	Chawla and Kalra (1976)
ORGANOPHOSPHATES			
Acephate	100.84	India	Sannaveerappanavar (1995)
Chorpyriphos	5.37	India	Sannaveerappanavar (1995)
Diazinon	14.25	India	Sannaveerappanavar (1995)
Dichlorvos	15.41	India	Sannaveerappanavar (1995)
Ethyl parathion	ND	India	Verma and Sandhu (1968)
		India	Deshmukh and Saramma (1973)
		India	Chawla and Kalra (1976)
Fenitrothion	ND	India	Chawla and Kalra (1976)
Malathion	ND	India	Chawla and Kalra (1976)
Methyl parathion	ND	India	Chawla and Kalra (1976)
	2726.13	India	Sannaveerappanavar (1995)
Monocrotophos	227.25	India	Sannaveerappanavar (1995)
	32.6-85.70 %	India	Chandrasekar and Regupathy (1996)
	78.42-83.90	India	Vastrad (2000)
Phosalone	109.94	India	Sannaveerappanavar (1995)
Profenophos	39.09	India	Sannaveerappanavar (1995)
Quinalphos	10-628	India	Saxena *et al*. (1989)
		India	Chawla and Joia (1992)
	7.58	India	Sannaveerappanavar (1995)

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Table 2 Cont.

	45.50-92.30	India	Chandrasekar and Regupathy (1996)
Triazophos	59.06	India	Sannaveerappanavar (1995)
CARBAMATES			
Carbaryl	108.01	India	Sannaveerappanavar (1995)
Methomyl	3-25	Japan	Hama (1992)
	9-10	Thailand	Kobayashi *et al.,* (1992)
	High	United States	Liebe and Savage (1992)
	2.05-780	United States	Shelton and Wyman (1992)
	1.1-5.78	United States	Zhao and Grafius (1993)
	High	China	Sun *et al.,* (1995)
	36.31	India	Sannaveerappanavar (1995)
	71.24-82.43 %	India	Vastrad 2000 (1996)
SYNTHETIC PYRETHROIDS			
Alphamethrin	>46138.07	India	Sannaveerappanavar (1995)
Cypermethrin	41-145	India	Saxena *et al.*(1989)
	26507.81	India	Sannaveerappanavar (1995)
	25.00	India	Raju and Singh (1995)
Deltamethrin	2814.07	India	Sannaveerappanavar (1995)
Fenvalerate	43-211	India	Saxena *et al.* (1989)
	2102-3569	China	Tang *et al.,* (1992)
	High	Japan	Murai *et al.,* (1992)
	98-100%	Thailand	Kobayashi *et al.,* (1992)
	82475%	United States	Yu and Nguyeu (1992)
	1.9 - 8.1%	South Korea	Lee *et al.,* (1993)
	Moderate	Mexico	Diaz *et al.,* (1994)
	27848.21	India	Sannaveerappanavar (1995)
	25.00	India	Raju and Singh (1995)
	38.9	China	Luo *et al.,* (1995)
	High	China	Sun *et al.,* (1995)
	197.4%	China	Zhu *et al.,* (1995)
	66.70-100%	India	Chandrasekaran and Regupathy (1996)
	38-581%	South Korea	Chung *et al.,* (1997)
	High	New Zealand	Cameron *et al.,* (1997)
	66.70-100%	India	Chandrasekaran and Regupathy (1996)

Table 2 Cont.

	71.19-87.36 %	India	Vastrad (2000)
COMBIPRODUCTS			
Profenophos + Cypermethrin	88.01	India	Sannaveerappanavar (1995)
TERTIARY AMINES			
Cartap hydrochloride	28.1%	Japan	Adachi and Futai (1992)
	3-25%	Japan	Hama (1992)
	10-11%	Thailand	Kobayashi *et al.,* (1992)
	10.5-33.3%	South Korea	Lee *et al* (1993)
	Moderate	China	Shuai *et al* (1994)
	9.1%	South Korea	Cho and Lee (1994)
	3.75	India	Sannaveerappanavar (1995)
	17.9-52.4%	India	Chandrasekaran and Regupathy (1996)
	10.89-20.63 %	India	Vastrad (2000)
BT PRODUCTS			
	112.5%	Malaysia	Syed (1992)
	10-50%	Japan	Hama (1992)
	60%	Japan	Morishita *et al.* (1992)
	11.7-41.7%	Japan	Adachi and Futai (1992)
	2-211%	US	Shelton and Wyaman (1992)
	10%	Thailand	Kobayashi *et al* (1992)
	1.1-41.1%	China	Zhao *et al* (1993)
	320.7-461.6%	US	Shelton *et al* (1993)
	24%	South Korea	Cho and Lee (1994)
	55.4-118.3%	Taiwan	Kao and Cheng (1994)
Bt. kurstaki	1.90-15.30	India	Sannaveerappanavar (1995)
	624%	US	Perez *et al* (1995)
	50%	Spain	Martinez *et al* (1995)
	20%	US	Liu *et al* (1996)
	405-1125.7%	US	Perez and Shelton (1996)
	20%	Hawaii	Liu *et al* (1996)
	3-14%	Malaysia	Muhammed *et al* (1996)
	27-42%	Taiwan	Adachi and Grey (1996)
	4.32-19.50%	Central America	Perez and Shelton (1997)
Biobit	8.00	India	Sannaveerappanavar (1995)
Dipel 8L	3.8-7.17%	India	Vastrad (2000)
Bt aizawai	1.90		Sannaveerappanavar (1995)

ND: Not determined.

resistance and control failures. In some areas, economic production of crucifers has become increasingly difficult (Raju, 1996; Regupathy, 1996). Diamondback moth has a long history of becoming resistant to every insecticide used extensively against it. Studies of DBM's resistance to insecticides have indicated the presence of three possible mechanisms:

i)   Reduced chemical penetration
ii)   Enhanced activity of detoxification enzymes,
iii)   Lower sensitivity of the target site.

Insecticide resistance and control failure are now common in tropical climate of India and elsewhere. Factors that influence the development of resistance in diamondback moth include high fecundity and reproductive potential (Justin *et al*.1989), rapid turn over of generation (Jayarathnam, 1977), long growing season and frequent insecticide application.

In India, the first record of insecticide resistance was made in 1966 when DDT and ethyl parathion failed to control DBM around Ludhiana, Punjab (Verma and Sandhu, 1968). Later resistance to several organochlorines and organophosphate insecticides in Haryana was reported by Verma *et al*. (1972). Deshmukh and Saramma (1973) also observed the populations of DBM collected from Jalandhar were less susceptible to DDT and ethyl parathion than those found in Ludhiana which confirmed the earlier reports. Within three years, resistance had extended to multiple insecticides including fenetrothion and malathion in major vegetable growing areas of Punjab (Chawla and Kalra, 1976). However, there was no report of DBM resistance against synthetic pyrethroids up to 1988. Saxena *et al*. (1989) determined the resistance level in field populations of DBM collected from different regions of the country. The pest has developed resistance levels of 145 fold against cypermethrin at Panipat, 178 fold against fenvalerate at Ranchi, 191.76 and 115.52 fold against deltamethrin at Delhi and Bangalore, respectively. A high degree of quinalphos resistance was detected in all the populations tested. Higher levels of resistance to synthetic pyrethroids were also reported from Punjab (Mehrotra, 1990; Chawla and Joia, 1991, 1992). In another study, Chawla and Joia (1992) found that populations from Amritsar, Jalandur, Ludhiana and Hoshiapur of Punjab had developed about 10 fold resistance when compared with LC_{95} values of quinalphos. Raju and Singh (1995) observed 25-fold resistance to cypermethrin and fenvalerate and 5 fold resistance to endosulfan. Joia and Chawla (1995) and Joia *et al*. (1996) have reported current status of DBM resistance to quinalphos (70X), fenvalerate (2700X) and cypermethrin (2880X) and cross-resistance among insecticides with varying modes of action.

Sannaveerappanavar (1995) worked out the diagnostic doses for several insecticides based on the LD/LC_{50} values for the 50th generation of a field collected population maintained in the laboratory under insecticide free conditions. Varying levels of (1.90 to 46138.07 fold) resistance to 23 insecticides has been reported from Bangalore. According to Rabindra *et al*. (1995), DBM population from different parts of Tamil Nadu exhibited differential susceptibility to fenvalerate, monocrotophos, chlorpyriphos and *B. thuringiensis* (*Bt*) indicating that some population were already on the road to resistance selection. Development of high

level of resistance a few years after the introduction of synthetic insecticides was reported by Chandrasekharan and Regupathy (1996a). The resistance level varied from 8.20 to 85.7% to different insecticides from various locations.

Chandrasekaran and Regupathy (1996b) used F26 laboratory reared population without exposure to insecticides and fixed discriminating doses for quinalphos, fenvalerate, carbosulfan, monocrotophos and cartap hydrochloride by different bioassay methods using third instar larvae. Of the different methods, the vial residue bioassay was preferred and used for assessing resistance levels in DBM population. For field monitoring of *Bt* resistance, use of a discriminating dose of 188 ppm ai/l was also suggested (Chandrasekaran and Regupathy, 1996c). Using the vial method, insecticide resistance to DBM was monitored at different locations in Tamil Nadu. High frequency of resistance was noticed to fenvalerate and quinalphos followed by monocrotophos, cartap hydrochloride and carbosulfan irrespective of locations. Surprisingly, resistance to carbosulfan, which was not yet available in the market for use at the time of reporting was also observed at low but significant level which might be due to the earlier use of carbofuran indicating that carbofuran selection extended to the pro-insecticide carbosulfan (Renuka and Regupathy, 1996). Possibility of existence of tolerant (resistant) populations of *P. xylostella* to *B. thuringiensis* sub sp. *kurstaki* in Delhi and Punjab populations in comparison to Karnataka population was recorded (Gujar *et al*. 1999).

Nirmal and Singh (2000) from Andhra Pradesh reported higher degree of resistance to fenvalerate followed by cypermethrin in both topical application and leaf residue bioassay methods They also suggested that leaf residue bioassay is more advantageous to use than topical application.

Muthugouder *et al,* (2000) developed base line susceptibility of *P. xylostella* against *B. thuringiensis* var. *kurstaki* (Biobit[R]) collected from 7 states in India spread over a distance of about 3000 km using cabbage leaf disc bioassay technique. Results showed an increase in LC_{50} from 2.76 to 5.28 mg ai/l and also reported the possibility of development of resistance to *B. thuringiensis* under field conditions. Resistance monitoring with discriminating dose in cabbage growing areas(Dharwad, Belgaum, Haveri and Bidar) of North Karnataka indicated that resistance was in the following order : Fenvalerate> monocrotophos> methomyl> endosulfan> cartap hydrochloride> B.thuringiensis (Vastrad 2000). He also evaluated selected IRM components *viz.,* insecticide with novel chemistries, oil combinations and biopesticides (*B. thuringiensis* var *kursitaki*) in the farmers field at Dharwad. Thiodicarb 75 WP (0.15%) and lufenuron 5 EC (0.005%) emerged as the most promising insecticides for managing resistant field population of DBM. Reduction of larval population in the plots treated with these chemicals ranged from 57.13 to 99.99% and yields were also maximum.

Resistance management (RM) entails the amelioration of the evolution of genetic adaptation to pesticide selection. The four basic principles of RM are to diversify mortality mechanisms, to reduce selection pressure, to manage susceptibility and to monitor and predict resistance development. RM is strategic feature of IPM and supports the idea of minimizing adverse pesticide effects on

society and the environment. RM objectives include decreasing chemical inputs, while increasing the products use life. Following are some of the Resistance management components:

- Resistance monitoring for effective decision-making.
- Crop rotation with non-cruciferous crops.
- Avoid unnecessary sprays of insecticides by observing ETL.
- Growing trap crops.
- Use of insecticide mixtures is not desirable.
- Always rotate different groups of insecticides.
- Use of botanicals (neem) and bio-pesticides (*Bt*)
- Conservation and/or augmentation of *D. semiclasum* and *C. plutellae.*
- Use selective insecticides to encourage natural enemies activity.
- Synergies pyrethroids with sesamum oil and honge oil 0.2% to manage resistant population.

9.   Integrated Approach

The information on the evaluation of different IPM modules is very scanty. However, based on extensive studies conducted on the population dynamics of DBM on cabbage, Jayarathnam (1977) recommended spraying of crop with an organophosphorus, insecticide only when economic threshold was reached. He also recommended sprinkling 5% jaggery solution in order to encourage the activity of predatory ants like *Tapinoma melanocephalaum, Pheidole* sp. and *Camponotus sericeus.* Further, the removal of old leaves of cabbage (where 60% of pupation occurs) is also advocated as one of the cultural practices likely to reduce incidence of the pest. Nagarkatti and Jayanth (1982) found parasitism by *C. (=Apanteles) plutellae* showing a clear density dependent relationship with the host during rainy and winter seasons at Bangalore. Hence, it is suggested to spray a suitable insecticide relatively safer to this parasitoid. Since, the population of parasitoids is low during summer moths, inundative release of *C. plutellae* to maintain the pest below economic injury level has been recommended.

In recent years, increased level of infestation of leafwebber, *C.binotalis,* has been recorded on cabbage. Along with DBM (Nagarkatti and Jayanth, 1982), the late larval instars of *C. binotalis* prefer to feed on primordia, which resulted in either aborted heading

or multiple heading (Srinivasan, 1984). Recognizing potential need for the development of a suitable management strategy effective against both these lepidopterans, Srinivasan (1984) suggested monitoring of low damage threshold on wrapper leaves of cabbage after giving a blanket spray to protect the primordia with phosalone at 0.07%. phosalone is recommended in view of its effectiveness against both lepidopterans and its relative safety to important natural enemies of the pest complex on cabbage. The adoption of visual damage threshold also results in considerable reduction in number of spray applications of phosalone (Srinivasan, 1984). Superimposition of damage threshold on the inter-crop combination of one row of cabbage and one row of tomato (cabbage planted 30 days later than tomato) is also an effective alternative approach to reduce the incidence of both pests and increasing cabbage yield significantly. Sirnivasan and Moorthy (1992) reported that planting of paired mustard rows at either end of 25 cabbage rows is the most promising planting pattern for the successful management of DBM. The first mustard row is sown 15 days prior to planting and the other is sown 25 days after. The inter-cropped cabbage is sprayed with 0.1% dicholrvos sprays starting from 15 days after sowing at either 10 or 15 days intervals depending on population pressure.

The farmer's practice was compared with a recommended integrated pest management for controlling *P. xylostella* in cabbage. Fewer sprays were applied with IPM strategy (8 Vs 25) and both yield (+60%) and returns (+152%) were markedly higher with the IPM strategy reported by Nataraju *et al*. (1997). Reddy and Guerrero (2000) from Karnataka reported the utilization of *C. plutellae* (2,50, 000 adults/ha), *C. carnea* (2500 eggs/ha), nimbecidine (625 ml/ha), *Bt* (500ml/ha) and phosalone (2.80 l/ha) in the IPM programme based on pheromone trap catch threshold of 8 moths/trap/night.

Yield and value of marketable cabbage were highest in the resistant variety treated with pyrethroid insecticide had the lowest marketable yield and value and the highest loss of marketable yield. Inter cropping was not reliable in controlling DBM, but an economic justification for planting the comparison crop was demonstrated. It is concluded that host plant resistance complimented by B. thuringenesis were suitable components in an IPM programme for DBM management in Jamaica (Ivey and Johnson, 1998).

Two IPM modules (Biointensive and adaptable) in comparison with recommended package of practice against DBM and other defoliators of cabbage has been evaluated. The adaptable module (Five sprays in sequence – synthetic insecticide – fipronil 0.0005%, *Bt* 0.02%, and neem oil 0.00015% + mustard as a trap crop on which dichlorvos was sprayed at 10-15 days interval) was found best in reducing the pest load on crop, controlling the pests, increasing the yield tremendously and conserving the natural enemies in crop ecosystem as well. This module recorded highest benefit cost ratio of 4.17.

10.   References

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11

Biocontrol of Nematode-Borne Diseases in Vegetable Crops

Geeta Saxena

ABSTRACT: Losses caused by plant parasitic nematodes to vegetable crops is a matter of grave concern. Biological control of nematodes is the use of microbial agents such as bacteria and fungi that reduce nematode population by their antagonistic behaviour. Fungi continuously destroy nematodes in virtually all soils because of their constant association with nematodes in the rhizosphere. *Pasteuria penetrans* has been the subject of intensive study as a promising biological control agent of nematodes. A large number of nematophagous fungi are known to trap or prey on nematodes, but the most imporant genera include *Arthrobotrys, Monacrosporium, Nematophthora, Hirsutella, Verticillium and Paecilomyces.* Application of some of these fungi in vegetable crops has given interesting results. Integrated nematode management can be realized by variety of techniques including predators and parasites, genetic resistance of host, soil amendment with organic mater and other cultural practices. Few commercial preparations of these fungi are also available in the market.

1.   Introduction

Nematodes are tiny thread like eleworms. Many are free-living saprophytes found in the soil that live and feed on decaying plant and animal matter. Other soil-borne nematodes are plant parasites which are small microscopic roundworms attacking the roots of the plants. Many different genera and species of nematodes are important to crop production in the world, most important of them is vegetable crops. The host range of plant parasitic nematodes include most, if not all of the commercially grown vegetables. Many of the plant parasitic nematodes predispose plants to infection by fungal or bacterial pathogens or transmit viral diseases which contributes to additional yield reduction.

Plant parasitc nematodes are limiting factor in agricultural production in many parts of the world. Their world wide distribution, extensive host range, association with fungi, bacteria and viruses in disease complex presents a very challenging problem damaging world's food supply. Although, the use of modern nematicidal chemicals provide effective control of plant parasitic nematodes, in some countries, the fumigants DBCP (1,2 dibromo– 3 chloropropane) and ethylene dibromide have been removed from the market because of health hazards and environmental pollution problems. With the increasing cost of testing the safety and feasibility of pesticides, the development of new nematicides has almost ground to a halt, which necessitates the use of additional non-chemical means of nematode control. Biological control will play an increasing role in practical nematode control in the future.

Biological control has been defined as the use of natural or modified organisms, genes or genes product to reduce the effects of undesirable organisms such as crops, trees, animals, beneficial insects and micro organisms. Biological control agents are natural enemies such as predators, parasites and competitive antagonists of crop pests which are either introduced into an area or augmented in its natural surrounding to enhance its establishment and control potential. Biocontrol is thus the use of one living organism to control another, the latter being a pest. In biological control an exotic beneficial organism is introduced against pests into a new area and becomes permanently established. Biocontrol agents have gained considerable grounds in crop protection due to their various positive attributes including specificity, lack of toxicity to non-target organisms and absence of any residual effects (Mukerji and Garg, 1988a, b).

Biological control of nematodes concerns microbial agents such as bacteria and fungi that are nematophagous or antagonistic to nematodes (Kerry *et al.,* 1993). Natural enemies of nematodes have been identified that may be parasites/pathogens, predators, competitors or antagonists. Many of these natural enemies occur together in soil and in the rhizosphere. Cultural methods such as crop rotation and soil amendments have been used to encourage the indigenous antagonistic rhizosphere microflora that may reduce nematode damage and populations (Kloepper *et al.,* 1991). Different nematode-trapping

fungi, endoparasitic fungi and parasites of cyst and root-knot nematodes predominate at different stages in the soil or life cycle of nematodes. Nematode-trapping fungi differ in their dependence on nematodes as a source of nutrition and they can live saprophytically in soil. They may form traps spontaneously or in response to the presence of nematodes (Jansson and Nordbring-Hertz, 1980). Their trapping activity depends on the nutrient status of the substrate (Cooke, 1962a, b). They are widely distributed in various habitats and all species of nematodes are trapped (Gray, 1987, Saxena and Mittal, 1997).

Plant parasitic nematodes especially root knot nematodes cause enormous damage to crops, especially vegetable crops. Root-knot nematodes being endoparasites, complete their entire life cycles in roots or soils. It leads to formation of knotted, swollen, deformed and stunted roots. Root-knot nematode disrupts the normal processes of plant root growth and its soil exploration capacity for both water and nutrients. It is an econimically important crop pest. All major field crops, vegetable crops, certain cash crops, ornamentals and grass plants are susceptible to attack by one or more species of root-knot nematodes. Root-knot nematode *Meloidogyne* spp. pose serious threat to commercial vegetable production in tropical and subtropical countries, where intensive cropping practices and suitable climatic conditions favour alarming population rise of nematodes. Losses caused by root-knot nematode in vegetable crops like tomato, onion, carrot, cabbage, celery, egg plant, spinach, beans, cucurbits and okra range from slight to total.

2.   Major vegetable crops having nematode diseases

Nematodes cause severe diseases of major vegetable crops (Table 1) all over the world. They feed in the intercellular spaces and cause disintegration of the cells. They enter the plant through wounds, stomata or water-pores. They cause swelling of the stems, irregularities in branching, deformation of the leaves, suppression of blossoms, and galls on/of the roots.

TABLE 1
Nematode types attacking vegetable crops.

Vegetable Crops	Nematodes infecting them
Cabbage-(*Brassica oleracea* var.*capitata*)	*Tylenchorhynchus brassicae, Heterodera* cruciferae, Meloidogyne *incognita, M.arenaria, M.hapla, M.javanica.*
Carrot (*Daucus carota*)	*M. incognita, M. hapla*
Cauliflower (*Brassica oleracea* var. *botrytis*)	*Tylenchorhynchus brassicae*
Celery (*Apium graveolens* var. *dulce*)	*M. incognita*
Chard (*Beta vulgaris* var. c*icla*)	*M. incognita*
Chilli (*Capsicum frutescens*)	*M.arenaria, M.incognita, Helicotylenchus dihystera, Pratylenechus delattrei.*
Cowpea (*Vigna unguiculata*)	*Rotylenchus reniformis*
Cucumber (*Cucumis sativus*)	*M.hapla, M.incognita, Pratylenchus penetrans, Rotylenchus reniformis.*
Eggplant (*Solanum melongena*)	*Belonolaimus longicaudatus, Helicotylenchus dihystera, M.incognita*
Frenchbean (*Phaseolus vulgaris*)	*M.hapla, Pratylenchus neglectus, Rotylenchus reniformis*
Gourd (*Lagenaria siccraria*)	*M.hapla*
Horseradish (*Armoracia rusticana*)	*M.hapla*
Kale (*Brassica oleracea*, var. *acephala*)	*M. incognita*
Lettuce (*Lactuca sativa*)	*M.eloidogyne arenaria, M.hapla, M. incognita, Pratylenchus neglectus.*
Leek (*Allium porrum*)	*M.hapla*
Okra (*Abelmoschus esculentus*)	*Helicotylenchus dihystera, M.hapla, M.incognita, Pratylenchus delattrei*
Onion (*Allium cepa*)	*H.dihystera, Ditylenchus, Trichodorus allius, M.hapla, M.incognita*
Parsley (*Petroselinum crispum*)	*H.dihystera*
Pea (*Pisum sativum*)	*M.hapla*
Pepper (*Capsicum annuum*)	*M.hapla, M.incognita*
Potato (*Solanum tuberosum*)	*M.hapla, M.incognita, Globodera pallida, G.rostochiensis, Trichodorus*
Pumpkin (*Cucubita moschata*)	*M.hapla, M.incognita*
Radish (*Raphanus sativus*)	*M.hapla, M.incognita*
Rhubarb (*Rheum rhaponticum*)	*M.incognita*
Rutabaga (*Brassica napobrassica*)	*M.hapla*
Spinach (*Spinacia oleracea*)	*M. incognita*
Squash (*Cucurbita pepo*)	*M. incognita*
Sweet Potato (*Ipomoea batatas*)	*M. incognita*
Tomato (*Lycopersicon esculentum*)	*Helicotylenchus dihystera, M.hapla, M. incognita, M.javanica, Rotylenchus renifornis.*
Turnip (*Brassica rapa*)	*M. incognita*

3.   **Rhizosphere Interactions of Nematodes**

A complete knowledge of the ecology of soil borne pathogens, their survival and interactions with other microbes in the rhizosphere is imperative to achieving biological control. The zone of soil around roots that form the rhizosphere is a dynamic, complex, habitat that varies in space and time. All plant parasitic nematodes are obligate parasites and must enter rihzosphere to reach their host, but the time spent in the rhizosphere depends on their parasitic habit. Between 10% and 30% of the carbon assimilated by the plant is released into the rhizosphere (Lynch and Whipps, 1990). This supports microbial activity that is generally much greater than in the bulk soil. There may be several times more bacteria and fungi. The structure of the microbial community around roots is greatly affected by environmental conditions and plant species. The influence of these factors on biocontrol agents of nematodes is little understood.

The rhizosphere as an interface of soil and roots is a hub of microbial activity and a zone of special interest to plant pathologists (Katznelson, 1965, Rovira and Davey, 1974, Bowen and Rovira, 1992, 1999, Lynch, 1990, Kerry, 2000). The roots of living plants create a special habitat by virtue of their conditions and materials sloughed off during root growth (Lynch and Whipps, 1990, Rovira, 1969). Root exudates play an important role in the establishment and maintenance of rhizosphere microflora and microfauna (Gupta and Mukerji, 2002). In exchange, microbial metablites also contribute to the unique niche by stimulating or inhibiting associated microorganisms and plant root growth . The qualitative and quantitative variation in the microflora of the rhizosphere is directly related to root exudations which in turn depend upon plant type, age and development factors, foliar application and microbial interactions (Curl and Trulove, 1986, Bowen and Rovira, 1992). The microbial community of the rhizosphere consists of pathogenic, symbiotic and saprophytic microbes which affect the growth and development of plants. A multiplicity of associative and antagonistic interaction among microorganisms adds an extra dimension to the rhizosphere effect (Boosalis and Mankau, 1965, Bowen, 1991). Rovira *et al.* (1974) estimate that despite the large microbial populations in the rhizosphere, bacteria occupy <10% of

the root surface and fungal hyphal denisties are only 12-14mm m^{-2} root.

Most studies on the nematodes are focussed on plant parasitic nematodes, although >60% of the nematode community in the rhizosphere are bacteria feeding (Griffiths, 1989). In the root, nematodes prefer to occupy elongation zone, young lateral apices or injured tissue, out of which the former two are major exudation zones (Rovira, 1973; Barker and Davis, 1996). Interactions between nematodes and rhizosphere microorganisms are complex and often specific. Nematode-trapping fungi may attack both free living and plant parasitic nematodes and their densities in the rhizosphere may be much greater than those anticipated. Nematophagous fungi depend on nematodes for their nutrition. Nematode-trapping fungi have saprophytic mode of survival as well, but obligate parasites depend on nematodes and grow little outside their nematode hosts. Spores of some obligate paraisites like *Drechmeria cornospora* that adhere to nematode cuticle may affect the nematodes ability to recognize hosts and result in reduced invasion of roots. More than 150 species of fungi have been isolated from cysts, femals or eggs of cyst nematodes. Some fungi like *Verticillium chlamydosporium* are largely confined to the rhizosphere and are more abundant on nematode infected roots, the extent of colonization depends on the isolate of the fungus and on the plant species (De Leij and Kerry, 1991).

In rhizosphere, nematodes, plants and other microorganisms share a tripartite relationship. Nematodes are attracted to host roots after getting signals present in root diffusates and identify appropriate sites for penetration of the host and initiation of feeding (Perry, 1996). Nematodes bring about morphological and physiologial changes in plants thereby influencing the root exudates and diffusates which in turn influences rhizosphere microflora and fauna (Doney *et al.,* 1970). Kumar *et al.* (1995) showed that root exudates of cowpea can indirectly affect the nematode population by influencing the egg pathogenic fungi and other microorganisms. Rhizosphere microorganisms like bacteria, actinomycetes and fungi produce toxins and secondary metabolites having nematicidal properties (Devidas and Rehberger, 1992, Anke *et al.,* 1995). Importance of microbial metabolites in the nematode management in soil has attracted much attention.

4.   **Bacterial Antagonists**

Some bacteria are the most common parasites of plant-parastic nematodes. The nematode antagonistic bacteria are broadly of two types i) bacteria that are pathogenic to nematodes or the nematode disease producing bacteria, ii) bacteria whose secretions or metabolic products are harmful to nematodes or the nematode toxin producing bacteria.

4.1 Bacteria that are pathogenic to nematodes

The *Pasteuria* group of bacteria are hyperparasties of nematodes. There are three gram positive bacterial species, *Pasteuria penetrans*, *P. nishizawae* and *P. thornei*, based on difference in their host range and morphology. All are obligate parasites. The biology and ecology of these parasites has been reviewed by Sayre and Starr (1988) and Chen and Dickson (1998). *P. penetrans* which attack *Meloidogyne* species has been focus of research. Its life cycle is of 20-30 days at 30º C (Stirling, 1981) but it's isolates differ in their temperature optima for the development and attachment of the spore to the host. Cobb in 1906 was the first to observe a population of *Pasteuria* sp. Mankau (1975) designated it as *Bacillus penetrans.* Sayre & Starr (1985) grouped populations of these bacteria in the genus *Pasteuria. Pasteuria* spores have been observed on several groups of soil nematodes including rhabditids, aphelenchids, tylenchids, dorylaims and mononchids (Sturhan, 1985). There are 10 orders having 96 genera and more than 205 nematode species that are hosts of *Pasteuria* (Sturhan, 1988).

4.1.1 Host range
The populations of *P. penetrans* is genetically heterogenous with respect to host specificity (Channer and Gowen, 1992). Spores of *Pasteuria* sp. originating from a particular nematode host generally attach to toxonomically close species of the original nematode host (Inserra *et al.,* 1992). The attachment of endospore to the nematode cuticle may vary with geographical distribution of the bacterial population. Australian populations of *P. penetrans* were more host specific than populations from USA (Stirling, 1985). In UK an isolate of *Pasteuria* collected from juveniles of *Heterodera avenae* also adhered to the cuticles of *H.schactii, H.glycines, Globodera pallida, G. rostochiensis* and *Meloidogyne javanica* (Davies

et al., 1990). Spores of an isolate collected from *H.cajani* in Haryana do not adhere to *M.incognita* and *M.javanica* (Walia *et al.,* 1990), Walia *et al.* (1992) reported from agricultural fields of Haryana widespread occurrence of *Pasteuria* spp. on *Meloidogyne* sp. *H.cajani, H.mothi, Verutus mesoangusts, Mesodorylaimus japonicus, Discolaimus tenax, Xiphinema brevicolle, Paralongidorus sali, Ecumenicus monhystera etc. Pasteuria* spp. have been found adhering to nearly 200 nematode taxa but number of nematode species serving as hosts for its development, is not clear.

4.1.2.   Life Cycle :

The life stages of *Pasteuria* has been described by Mankau (1975) and Sayre and Starr (1985). The microcolonies of the bacterium break away from the colonised hypodermis and proliferate within the pseudocoelom of the nematode. As the nematode develops into adult, daughter colonies continue to form upon lysis of intercalary cells in the microcolony. Bacterium utilizes the body content and eventually quartets of developing sporangia fill the nematode pseudocoelom. They form doublets which form sporangia each producing single endospores. The onset of sporogenesis coincides with the initiation of reproductive phase in nematode. There is no or little egg production by the root-knot female and ultimately it turns into a carcass full of bacterial spores. The endospores are finally released into the soil. Endospores of *P. penetrans* are non motile and they are resistant to heat and desiccation (Oostendorp *et al.,* 1990) They may survive serveral years in soil (Chen and Dickson, 1998). Their dissemination depends upon the size of soil pore opening, tillage practices and soil invertebrates (Sayre and Starr, 1988).

4.1.3   Endospore Attachment:

The endorspores of *Pasteuria* adhere to the nematode cuticle in the soil. The extent of spore encumbrance is influenced by the population of bacterial endospores and nematodes (Davies *et al.,* 1991); temperature (Stirting *et al.,* 1990; Hatz and Dickson, 1992). The spores generally adhere to anterior or posterior part of nematodes in greater number (Sharma and Davies, 1996). The spore surface is covered with fine fibres (adhesins) that are involved in the attachment of spore to the nematode cuticle (Persidis *et al*., 1991). The surface of the spore is negatively charged and a balance between electrostatic and hydrophobic interactions is important in deciding the spore attachment to the nematode cuticle (Afolabi *et al.,* 1995). The N-acetyl glucosamine (Persidis *et al.,* 1991) and glucosyl residues on the spore surface attach to the lectins on the nematode surface (Davies and Dank, 1993). It has been found that several nematode surface coat components, such as carbohydrate residues, carbohydrate recognition domains and a 250 KDA antigen are involved in spore attachment of *P. penetrans* to the surface of *Meloidogyne javanica.*

4.1.4   Germination :

The endospore attached to the nematode cuticle germinates only after the root-knot nematode establishes feeding sites in the host root, and it takes about 8 days

after root invasion (Sayre and Starr, 1988). The germ tube of the endospore penetrates the cuticle probably by enzymatic action (Stirling *et al.,* 1986). After entering the hypodermal tissue, the germ tube develops into a dichotomously branched primary thallus. This colony gives rise to daughter colonies within the pseudocoelom.

4.2 Bacteria that produce nematode toxin

Several types of bacteria colonize the rhizosphere. The metabolic products of these bacteria affect plant parasitic nematodes along with other micro organisms. Some bacteria decompose the organic matter and the decomposition products such as hydrogen sulphide, ammonia and volatile fatty acids inhibit the nematode populations. Some plant growth promoting bacteria such as *Rhizobium, Bradyrhizobium* and hetertrophic bacteria like *Azotobacter* and *Azospirillum* have nematode antagonistic traits (Huang, 1987; Ramakrishnan *et al.,* 1996). The rhizobacteria regulate the nematode behaviour during the early root penetration phase of parastism. The nematode infection is reduced probably due to i) production of metabolites which reduce hatching and host attraction, ii) degradation of specific root exudates which control nematode behaviour (Sikora and Hoffman-Hergarten, 1993). Strains of *Bacillus thuringiensis (Bt)* are toxic to plant parasitic nematodes.

4.3 Bacteria as potential biocontrol agent

Sikora (1992) found that out of several rhizobacteria isolated, about 7-10% have antagonistic potential against cyst and root-knot nematodes. In greenhouse experiment, *Agrobacterium radiobacter* and *Bacillus sphaericus* cause 41% reduction in root invasion by *Globodera pallida* in potato. In field trials the blend of two bacteria caused a 31% and *B. sphaericus* alone 29% reduction in root invasion by the nematodes. Tuber yield increased by 18% and 22% in the field trials when tubers were treated with *A.radiobacter* or the blend, respectively (Racke and Sikora, 1992).

Jacq *et al.* (1977) found that a thermostable toxin of *B. thuringienis (Bt)* was toxic to populations of *Meloidogyne, Panagrellus* and *Aphelenchus* and prevented *Meloidogyne incognita*

larvae from forming galls on tomato roots. Zuckerman *et al.* (1993) reported that a strain of *Bt* (CR-371) caused significant reduction in galls on tomato in a greenhouse trial.

In recent years, *Pasteuria penetrans* has been the subject of intensive study as a promising biological control agent of nematodes. *P. penetrans* is the most specific parasite and has been found to exert effective control of root-knot nematode. Most of the biological control studies are on *Meloidogyne* spp. (Mankau, 1972). In root-knot nematodes, germination of the spore occurs about 8 days after an encumbered nematode enters a root and initiates feeding in the host (Sayre and Wergin, 1974). The nematode may be killed or reach maturity without producing eggs (Mankau, 1980). After the decomposition of the dead nematode, spores remain free in the soil until contacted by another nematode. Spores may live in the soil for at least 6 months and they are not affected by normal field temperature. *P. penetrans* appears to be perfectly synchronized with the root-knot nematodes development and physiology. The resistance of these long lived spores to heat and desiccation and their favourable compatibility with nematicides are characteristics well suited for their eventual practical use in field soils.

Application of *P. penetrans* in several greenhouse and microplot experiments, has consistently suppressed the nematode induced root galling and egg production with consequent enhanced plant growth (Zaki and Maqbool, 1992, Daudi *et al.,* 1990, Walia, 1994). In greenhouse experiment, air dried soil infested with spores of *P. penetrans* was planted with tomato seedings, to which 10000 root-knot nematode larvae was added. After 70 days, plants in the air dried spore infested soil were found healthier than plants in soil free of spores. Roots of these plants had considerably less nematode galling as compared to other treatments. Mankau (1975) found that spores of the bacterium orginating from *Meloidogyne* sp. reduced the recovery of *Pratylenchus scribneri* from soil planted with beans. Mankau (1980) found that populations of *P. penetrans* do not increase rapidly in field soil. Greenhouse experiments have shown that a population of *Meloidogyne incognita* can be destroyed by *P. penetrans* in a few generations of the host. In Florida it was found that when soil in outdoor plots were infested with *M. incognita* and *P. penetrans* and

planted with successive host crops less damage was caused by the nematodes to plants having *P.penetrans*. Walia and Dalal (1994) recorded 18-20% increase in tomato yield by treatment of *M. javanica* infested nursery soil with *P. penetrans*. At harvest of tomato crop about 18% and 50% of the juveniles in soil had spores adhering to their body in the treatments of 1×10^4 and 1×10^5 spores respectively.

Application of *Paecilomyces lilacinus* and *P. penetrans* together is more effective in controlling the root knot nematodes and enhancing the crop yield than application of either of the organisms alone on okra (Zaki and Maqbool, 1991) and egg plant (Zaki and Maqbool, 1992). *Verticillium chlamydosporium* and *P. penetrans* together gave 92% control of *M. incognita* population on tomato in pot experiments (De Leij *et al.,* 1992). The most significant evidence of the natural built up of *P. penetrans* and control of *Meloidogyne arenaria* is in peanut field in Florida (Oostendorp *et al.,* 1991).

Soil infested with endospores of *P. penetrans* can be applied in the field where nematode control is needed. The effective control of nematodes by *P. penetrans* is dependent on the density of spores per unit soil (Ciancio and Bourijate, 1995). Stirling *et al.* (1990) have attempted to predict the concentration of *P. penetrans* spores needed to control root-knot nematodes in a field. Their data suggest that spore concentration of 1×10^5 and 2.2×10^5 spores/gm soil respectively, would be required at 27°C to ensure that an average of 20 and 50 spores are attached per nematode. These concentration are indicative of the inoculum quantity based on limited data, thus need further verification.

The major problem in using *P. penetrans* as a biocontrol agent is that it is an obligate parasite and attempts to culture them *in vitro* have been unsuccessful. The use of this bacterium on a large scale will probably depend on commercial *in vitro* cultivation of the organism. Presently there are no techniques for mass production of spores for large scale field application. However, root powder consisting of *Pasteuria* infected nematodes serves as a good source of endospore inoculum. It is possible to treat small areas in the nursery beds, spot application and seed coating. Vegetable nurseries having *Pasteuria* infected nematodes may aid in gradual control of the root knot nematodes in the fields because the population densities of

P. penetrans increase gradually over time from the relatively low levels of spores initially. In can build up gradually the levels which may keep the nematode population below damaging levels in due course of time. The promising characteristics of *P. penetrans* justify its potential as biocontrol agent. Its major attributes are long viability of spores, resistance to heat and deciscation, persistence in field soils, non toxicity to plants, easy storage and demonstrable nematode control potential.

5.   Nematode-Destroying Fungi

Among the microorganisms that parastize or prey upon nematodes or reduce nematode populations by their antagonistic behaviour, fungi has important position and some of them have shown great potential as biocontrol agents. These fungi are called nematophagous or nematode-destroying fungi (Fig.1). These fungi continuously destroy nematodes in virtually all soils because of their constant association with nematodes in the rhizosphere. The nematophagous fungi have been placed into three categories, predatory, endoparasitic and parasites of root knot and cyst-forming nematodes.

5.1 Nematode-trapping fungi

Predatism in the world of fungi in one of the most fascinating phenomenon. Predators also known as nematode-trapping fungi have extensive hyphal system on the substratum that produce trapping devices along their hyphae at intervals for the capture of nematodes. The killing of nematodes takes place by adhesive or non-adhesive trapping devices.

5.1.1. Adhesive trapping devices:
Adhesive organs of capture have been categorized as hyphae, branches, knobs and nets (Fig.1). Adhesive hyphae are characteristics of Zygomycetes and have no cross walls in the active vegetative hyphae. Hyphae are either coated with adhesive along entire length or produce adhesive at any point in response to nematodes. A common example of such type of trapping device is *Stylopage hadra* (Drecheler, 1935) in which the hyphae are rather sparse and are characterized by an irregular branching. Adhesive branches are found mostly in Deuteromycotina. They are morphologically the simplest form of capture organs which are a few

cells in height. They arise as short laterals which are arranged in a fairly close array. In *Monacrosporium cionopagum* and *M. gephyrophagum,* there is a tendency to form simple two dimensional nets. Adhesive knobs are morphologically distinct cells, they are either sesile or produced at the apex of a slender stalk composed of one to three cells. The knob is separated from the support stalk by a septum. They are found in Deuteromycotina and Basidiomycotina. The best example is *Nematoctonus*. Adhesive nets are the most commonly occuring trapping device (Cooke and Godfrey, 1964). Net forming fungi are ubiquitous in occurrence. Nets vary from a single loop to complex multibranched, three dimensional networks as in *Arthrobotrys oligospora.* The adhesive is highly effective and the prey is held fast. The common examples are *Arthrobotrys, Dactylella, Dactylaria* and *Monacrosporium.*

5.1.2 Non-adhesive trapping devices

Non-adhesive devices are either non-constricting or constricting rings. Members of Deuteromycotina produce these types of rings on lateral branches arising from prostrate hyphae. They are three-celled-rings supported on a slender stalk. Non constricting rings are passive in their action. Prodcution of non-constricting rings is often associated with adhesive knobs as in *Dactylaria candida* and *D.lysipaga* (Drechsler, 1937). Constricting rings are the most efficient method of trapping nematode. They are produced in a similar manner as the non-constricting rings. The ring cells are sensitive all along the inner edges. When a nematode enters a ring, the friction of its body stimulate the ring cells to swell rapidly inwards. This constricts the body of the nematode. Such rings are reported from members of Deuteromycotina as in the case of *Arthrobotrys anchonia* (Drechsler, 1954) and *Dactylaria brochopaga* (Drecheler, 1937).

5.2 Endoparsitic fungi

In endoparsitic fungi, there is no extensive hyphal development outside the body of the host (Fig.1). In the case of lower fungi, only evacuation tubes and in higher fungi conidiophores and conidia are produced externally in the surroundings. The endoparasites exist in the form of spores which remain viable but dormant while in some, they are persistent and help to withstand adverse conditions. The spores after dissemination serve as infective agents. They adhere to the cuticle of the host or ingested by them, while flagellate spores encyst on host body and germinate. Endoparasites are found in many groups of fungi such as Chytridiomycetes, Oomycetes, Zygomycetes, Basidiomycotina and Deuteromycotina. Few example are *Catenaria, Myzocytium, Haptoglossa, Harposporium, Meristacrum, Drechmeria,*
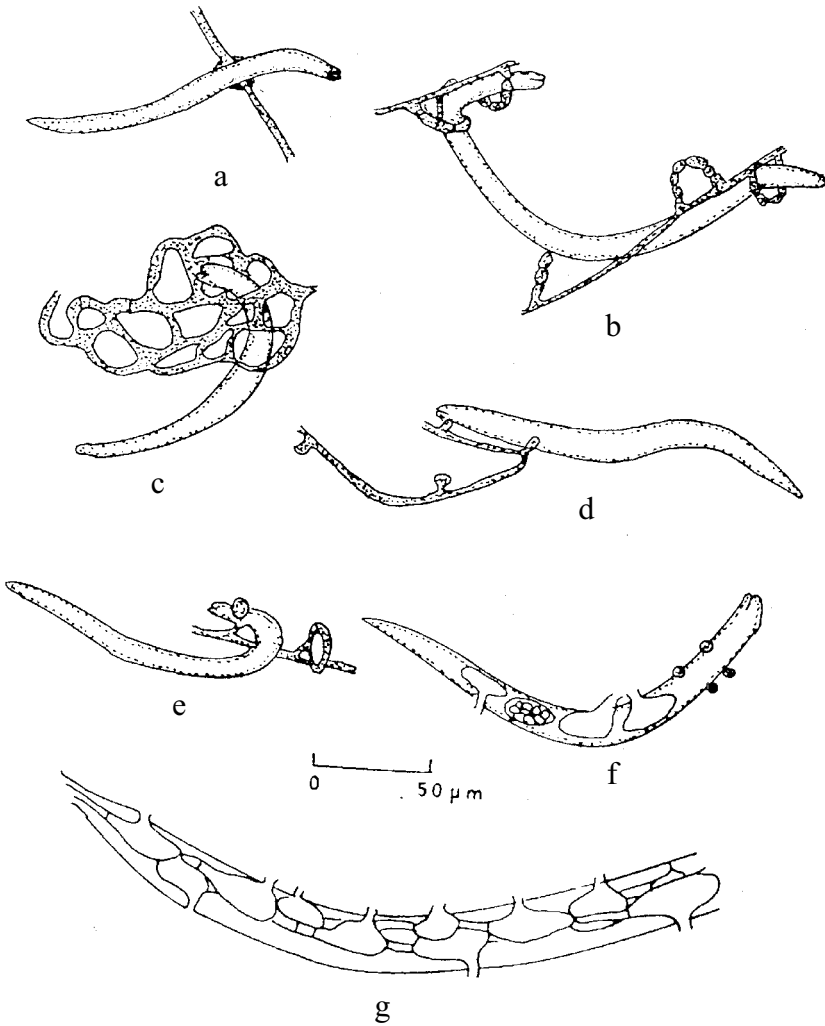
Fig. 1 : Predatory fungi showing different trapping devices. a. Adhesive hypha
of *Stylophage leiohpha* which has secreted adhesive to capture the nema-
tode; b. Adhesive branches of *Monacrosporium gephyrophagum* which
have united to form scalariform traps to capture nematodes; c. Namatode
captured in adhesive network of *Arthrobotrys oligospora.* d. Adhesive
knobs of *Monacrosporium parvicolle* capturing a nematode. e. A
namatode captured in constricting ring of *Arthobotrys brochopaga.*
f. Endoparasitic fungi. g. *Myzocytium papillatum* infected a nematode.
Full as well a empty zoosporangia are seen; g. A nematode full of zoospo-
rangia of *Catenaria anguillulae*. Empty sporangia with evacuation tubes
are seen after the release of zoospores.

Nematoctonus etc. The most studied endoparasites are *Catenaria anguillulae, Drechmeria coniospora, Hirsutella rhossiliensis* and *Verticillium balanoides*, although the most common fungus encountered among endoparasites with ingested spores is *Harposporium anguillulae* (Zop, 1888; Barron, 1969).

5.2.1 *Catenaria anguillulae*

Catenaria anguillulae is a widespread endoparasite, but a weak parasite (Barron, 1977). The zoospores of *C. anguillulae* are uniflagellate with a posterior whiplash flagellum, produced in zoosporangia inside the nematode body, escape through the tip of a solitary exit tube. Deacon and Saxena (1997) found that the zoospores were attracted to and encysted near the mouth, excretory pore and anus of nematodes. Cysts germinated within 20-60 minutes by a narrow germ tube at the site of adhesion. The germ tube penetrated a nematode and formed a vesicle inside the host. Rhizoids or assimlative hyphae developed trom the vesicle or by growth of the germ tube tip. A consistent orientation (polarity) of zoospore encystment and cyst germination was found. Pathogenicity studies by Esser and Ridings (1973) added 13 genera and 9 species of nematodes to the host range of the fungus. The influence of temperature, pH, salt concerntration and nature of substrate on the incidence of C. *anguillulae* has been studied well (Sayre and Keeley, 1969). The antagonistic potential of different isolates of *C. angillula*e varied against *H. schachtii* (Voss *et al.,* 1992). *C. anguillulae* also infects nematode eggs after zoospores encyst on their surface and produce a germ tube that penetrates the shell (Wyss *et al.,* 1992).

The lipid layer was a barrier to infection by this fungus, but once penetrated other zoospores showed taxis towards the egg and swarmed on its surface. The observation provided supporting evidence that the lipid layer was the layer that affected eggshell permeability (Perry and Trett, 1986) *C.anguillulae* rapidly killed the embryo suggesting that toxins may be involved.

5.2.2 *Hirsutella rhossiliensis*

Hirsutella rhossliensis parasitises and kills nematodes *in vitro (*Jaffee and Zehr, 1985; Timper and Kaya, 1989). It parasitises high number of nematodes in growers fields (Lackey *et al.,* 1993). It produces spores that adhere to and penetrate the nematode cuticle and assimilate the body contents prior to the emergence and sporulation (Jaffee and Zehr, 1985). The fungus was always more effective in soil infested with juveniles than in soil infested with egg masses or cysts (Lackey *et al.,* 1994). *H. rhossiliensis* is a predominant parasite of potato cyst nematode G*lobodera pallida* (Velvis and Kamp, 1995). Detached conidia of *H. rhossiliensis* did not adhere to the juvenile cuticle, but newly formed conidia attached to conidiophores, adhered to and infected juveniles. *H. rhossiliensis* has been described as a natural suppressive factor of other nematode pests. It has a broad nematode host range which includes plant parasitic nematodes (Timper *et al.,* 1991; Tedford *et al.,* 1993). Field observations demonstrated that parasitisation

of nematodes by *H. rhossiliensis* is dependent on nematode density and natural epidemics of this fungus in populations of nematodes develop slowly and only after long periods of host density (Jaffee, 1992). The actual impact of *H. rhossiliensis* cannot be derived from the percentage of infected juveniles recovered by the method of centrifugal floatation, because most juveniles apparently disintegrate relatively quickly after infection. Rapid disappearance of nematodes in soil after infection by fungus was also reported for *Circonemella xenoplax* (Jaffee *et al.,* 1988). In the USA the fungus is a common parasite of *C. xenoplax* in peach orchards (Jaffee and Zehr, 1982) and suppressed the multiplication of this nematode in laboratory tests (Eayre *et al.,* 1983).

The value of *H. rhossiliensis* is its abidity to parasitize a broad range of plant parasitic nematodes including *Globodera, Heterodera, Meloidogyne, Praty lenchus* and *Ditylenchus* (Timper and Brodie, 1993). This fungus seems to be ecologically well equipped for infection of free-living juveniles in the range of soils which indicates its function in reducing root damage by juveniles of cyst and root-knot nematodes and other vermiform parasitic nematodes.

H. rhossiliensis is an obligate parasite in nature (Jaffee and Zehr, 1985; Jaffee, 1992). The life history of this fungus is simple (Jaffee *et al.,* 1990). The conidium surrounded by an adhesin and borne on a phialide attaches to the nematode. At 25°C, after 12 h a germ tube penetrates the cuticle directly beneath the conidium. After penetration it forms infection bulb, from which thin walled assimilative hyphae grow and ramify through the nematode body. Within 3 days, the nematode is completely filled with hyphae. In suitable condition the fungus grows from the nematode and produces new conidia on phialides. Tedford *et al* (1994) found that genetic variation among isolates is substantial but not variation in pathogenicity and morphology.

5.3   Parasites of cyst-forming and root-knot nematodes

Such parasites do not invade eggs or females by means of specialized structures such as traps or spores, but by the growth of vegetative hyphae with or without an appresorium like swelling. The role of these fungi as biological control agents has attracted much attention. Interactions between fungal antagonists and nematodes in agricultural soils have been known for many years (Mankau, 1980 a,b, Barrron, 1977, Drechsler, 1941).

5.3.1 Parasites of cyst forming nematodes

In the past few years there has been increasing interest in the pathology of cyst-forming nematodes. Parasites of the nematode genera *Heterodera* and *Globodera* received attention as biocontrol agents. In general fungi colonising eggs and cysts are more numerous than those parasitizing females (Gintis *et al.,* 1983).

(i.) Parasites of females

Parasites of female nematodes generally fall into two groups; obligates and non-obligates. The obligate parasites can invade females within a few days of exposure on root surfaces.

Catenaria auxiliaris parasitizes the females of *Heterodera avenae, H. glycines* and *Globodera rostochiensis* (Kerry, 1975, 1987, Kerry and Crump, 1977; Kerry and Mullen, 1981, Crump *et al.,* 1983). Kerry (1975) suggested that nematode populations decline because females on the root system either fail to form cysts or produce fewer eggs than expected. He observed four species of nematode parasitic fungi that were widespread in cereal fields infested with *H.avenae viz. Verticillium chlamydosporium, Cylindrocarpon destructans, Tarichium auxiliare* and an *Entomophthora* like fungus. Kerry and Crump (1977) found the *Entomophthora* like fungus to be capable of infecting *H.goettingiana, H.trifollii, H.carotae, H.schachtii, H.cruciferae* and *H.avenae.*

Nematophthora gynophila is an oomycetous fungus with biflagellate spores. This fungus was found to be widespread in Britain. The fungus completes its life cycle in the cereal cyst nematode within 5 days at 13°C (Kerry and Crump, 1980). The zoospores require water filled soil pores for infection which is limited to periods following rainfall.

(ii) Parasites of eggs

Eggs parasites are potential biocontrol agents of nematodes. Nematode eggs of the group Heteroderidae are vulnerable to attack to parasites. Once in contact with the cyst or egg masses, parastic fungi grow rapidly and eventually parasitize all of the eggs that are in an early embryonic stage of development. Most of these organisms are known to have chitinolytic activities.

Tribe (1977) reviewed the relationship of fungi to cyst pathology and listed *Verticillium chlamydosporium* as a major pathogen of eggs within *H.schachtii and H.avenae* cysts. Although *V.chlamydosporium* is mainly an egg parasite, it may attack virgin females. Diseased females form small cysts that contain few eggs. This fungus was recorded from 76% of soils during a survey of fungal parasites of *H.avenae* in southern England (Kerry 1975). Kerry (1981) recorded that *V.chlamydosporium* attacked the eggs of *H.avenae* and reduced their populations to non-damaging levels in a wide range of soils in Europe. Eggs of all cyst nematode species tested are susceptible and parasitized females formed small cysts containing few eggs. Crump *et al* (1983) observed *V. chlamydosporium* to be the most frequent parasite of the eggs and larvae of *H. schachtii.* It was responsible for maintaining nematode populations below the economic threshold (Kerry *et al.,* 1982: Kerry and Jaffee, 1997). It reduces fecundity and kills many eggs resulting in a decline in nematode population. Kerry *et al. (1986)* tested six strains of *V. chlamydosporium* isolated from *H. avenae* eggs and found that they varied significantly in their pathogenicity to *H. avenae eggs.* Similar results were found in *H. schachtii* eggs using six other strains of this fungus (Irving and Kerry, 1986).

Cylindrocarpon destructans has been recorded in cyst of *H. schachtii* (Bursnall and Tribe, 1974). It is the most frequently encountered species associated

with *Globodera pallida,* the potato cyst nematode. Crump (1987) isolated *C.destructans* from infected females of *H.schachtii* and *H.avenae* on sugar beet roots in two soils. The potential value of *C.destructans* has yet to be established.

5.3.2 Parasites of root-knot nematodes

Root knot disease caused by *Meloidogyne* spp. is a matter of grave concern as it affects several economically important crop plants.

Verticillium chlamydosporium is a widespread faccultative parasite of root-knot nematodes (*Meloidogyne* spp.) and is frequently found in soils that are naturally suppressive to populations of cyst nematodes (Kerry, 1995). The colonisation of the rhizosphere by the fungus is essential for the control of root-knot nematode populations (De Leij and Kerry, 1991) but the extent of colonisation is greatly affected by the host plant (Bourne *et al.,* 1996). Morgan Jones *et al* (1981) reported *Verticillium chlamydosporium* as a parasite of females and eggs of the root-knot nematode *Meloidogyne arenaria* in the soil of an Alabama peanut field. Godoy *et al.* (1983) isolated V. *chlamydosporium* from eggs of *M.arenaria.* Results from a grenhouse test suggested that *V. chlamydosporium* is capable of controlling *M.arenaria* effectively.

Few studies have examined the soil and environmental factors which may affect growth of the fungus in the rhizosphere and its survival in soil. The proliferation of *V.chlamydosporium* is greater in organic soils than mineral soils, (De Leij *et al.,* 1993). It proliferates in soil and in the rhizosphere where it parasitizes nematode eggs which are laid in egg masses on the root surface. The isolates differ in their pathogenicity to root knot nematode eggs, their ability to grow in the rhizosphere and in production of chlamydospores, which are preferred source of inoculum (Bourne *et al.,* 1994), as well as their temperature optima (Kerry *et al.,* 1986). In addition the host plant has significant effect on the growth of the fungus in the rhizosphere (Bourne *et al.,* 1996). Root-knot nematodes generally cause more damage in mineral soil and such soils support limited growth of *V.chlamydosporium* (De Leij *et al.,* 1993). Environmental factors also affect the survival and proliferation of the fungus in soil.

Although its capacity to penetrate females and eggs and to increase its biomass within these structures is well documented, the precise mode of action of its parasitism has not been elucidated. Likewise little is known of its physiology. Two possible types of activity, operating separately or in combination, are thought to be involved in the parasitism of cysts and eggs by fungi. It seems likely that diffusable fungal toxins can deleteriously affect eggs and even lead to premature death of the larvae. Exoenzymes, which might affect the permeability of egg shells, could operate in tandem with mycotoxins to predispose eggs to potential invasion. One or more of these factors might render the females and hatched larvae vulnerable to infection (Morgan Jones *et al.,* 1983). *V.chalmydosporium* is able to colonise the egg masses of root-knot nematodes which contain antibiotic substances (Orion and Kritzman, 1991), shows some tolerance to antifungal compounds and possesses protease enzymes that attack the outer layer of the nematode egg shell (Segers *et al.,* 1994). These characteristics suggest that *V. chlamydosporium* should be an effective competitor in soils.

Paecilomyces lilacinus is a typical soil-borne fungus and seems to be relatively common and ubiquitous in the tropics and subtropics. It is a soil hyphomycetes which has demonstrated tremendous potential as biocontrol agent of nematodes (Jatala, 1985). *P. lilacinus* infecting eggs of *Meloidogyne incognita acrita,* was discovered in Peru when placed in soil containing nematode infected plants, 70 to 90% of the eggs of *M.incognita acrita* and *Globodera pallida* were infected after one month (Franco *et al.,* 1981, Jatala *et al.,* 1979). In field trials against *M.incognita*, parasitizing potato 86% of the egg masses were infected and 55% of the eggs in those masses were destroyed (Jatala *et al.,* 1980). In a 3 year trial with *P.lilacinus,* added only in the first year crop damage by *M.incognita* was reduced each year. The root galling index on the original crop was severe, but was reduced to moderate amounts on successive crops (Jatala *et al.,* 1981). Godoy *et al.,* (1983) indicated that this fungus was effective in reducing *M.arenaria* infestations. Cabanillas *et al.,* (1988) observed the absence of root-galling and giant cell formation in tomato roots inoculated with nematode eggs infected with *P.lilacinus.* Microplot experiments were conducted to evaluate the effects of inoculum level and time of application of *P.lilacinus* on the protections of tomato plants against *M.incognita.* The efficiency and adaptability of *P.lilacinus* in effectively controlling several pathogenic nematodes has been studied under different climatic and soil environmental conditions all over the world (Noe and Sasser, 1995; Khan and Hussain, 1986, Morgan-Jones *et al.,* 1984, Cabanillas *et al.,* 1988; Cabanillas and Barker, 1989; Candamedo-Lay *et al.,* 1982; Davide and Zorilla, 1983; Roman and Rodriguez-Marcano, 1985; Villanueva and Davide, 1984). There are several reports of the use of *P.lilacinus* in the control of root-knot causing nematodes of economically important plants; (Khan and Hussain, 1986; Morgan-Jones *et al.,* 1984, Saxena and Mukerji, 1988: Saxena *et al.,* 1991). Recent experiments regarding *P.lilacinus* infestations in fields in Peru, Panama, Phillipines, Puerto Rico, Malaysia and United States indicate a significant role of the fungus in its potential as a biocontrol agent for the control of *M.incognita.* The extent of its activity was found better than any of the commonly used nematicides (Candanado *et al.,* 1983, Jatala, 1983).

As a potential biocontrol agent, *P.lilacinus* appears to have a number of characteristics. Most reports indicate that it is a good competitor in most soils, particularly in warmer regions (Domsch *et al.,* 1980). Its optimum growth temperature is 26-30° C. It readily produces abundant inoculum in the form of long chains of conidia. It acts as a chitin degrader having proteolytic properties (Endreeva *et al.,* 1972; Wainwright and Pugh, 1974; Borout, 1960). It has the ability to compete effectively, colonize natural soils and to exercise a degree of control against *Meloidogyne.* It has the greatest potential of all the parasites of eggs and cysts of phytonematodes, for application as biocontrol agent in subtropical and tropical agricultural soils. Due to these properties this fungus has been the subject of many recent investigations.

The infection of root knot and cyst nematode eggs by *P.lilacicus* has been studied using TEM and SEM (Morgan Jones *et al.,* 1984; Lopez-Llocra and Claugher, 1990; Segers *et al.,* 1996). Morgan Jones *et al.,* 1984 by ultrastructural

studies found that *P.lilacinus* is capable of parasitizing and thus destroying both the eggs and larvae of *Meloidogyne.* This fungus infects by producing an appresorium laterally or at the tip of hyphae growing across the egg surface, although this may not always be essential (Dunn *et al.,* 1982). Penetration of the egg shell is considered to be due to both enzymatic degradation and physical forces. Since the egg shell of *M.incognita* has been reported to be made up mostly of protein and chitin (Bird and Mc Clure, 1976), chitinase activity has been recorded in all egg parasitic fungi tested. The egg shell of most nematodes has an outer vitelline membrane and all have chitin and lipid layers which differ in thickness (Perry and Trett, 1986). Bonants *et al.* (1995) partially characterized a subtilisin like serine protease in *P.lilacinus*. Vitellin and the presence of eggs induced the enzyme when the fungus was grown on minimal media and the enzyme was repressed in the presence of glucose. Immature eggs exposed to the enzyme failed to develop but it increased the hatch of more mature eggs.

6   AM Fungi In Nematode Control

AM/VAM fungi are widespread in nature and are known to enhance growth and yield of plants (Dixon *et al.,* 1997, Mukerji *et al.,* 1997, 2000). AM (arbuscular mycorrhizal fungi) have stable plant-fungus interaction. Colonization of the root by AM fungi imparts the protection against disease by pathogens to the plant. The parasitization of plants by nematodes (mainly endoparasites) can be influenced by establishment of AM fungi. AM and nematodes due to their dependence on a host plant share a common habitat *i.e.* roots and thus are intimately associated. Both AM and nematode exert a characteristic, but opposite effect on plant growth (Hussey and Roncadori, 1982; Schenck, 1983). A better understanding of AM fungi is required concerning ecophysiological parameters contributing to effectiveness and the mechanism involved (Hooker *et al.,* 1994). Symptoms of nematode infection are generally reduced and often nematode populations are also reduced (Hussey and Roncadori, 1978).

Interactions between root-knot nematode *Meloidogyne incognita* and AM fungi have been primarily evaluated on tomato (Sikora, 1978; Thomson-Cason *et al.,* 1983; Bagyaraj *et al.,* 1979). Sitaramaiah and Sikora (1982) showed that inoculations of tomato transplants with *Glomus fasciculatum* significantly reduced root penetration by juveniles and the development of *Rotylenchus reniformis* compared

to controls. Fewer eggs per egg mass were produced on inoculated plants. Suresh *et al.* (1985) found that number of galls formed by *M.incognita* on tomato was significantly lower in AM inoculated tomato plants. However, the AM colonization did not prevent the penetration by the larvae. Cooper and Grandison (1986) studied the interaction of AM fungi with root-knot nematode *M. hapla* on tomato cultivars. Al-Raddad (1995) showed that preinoculations of *Glomus mosseae* significantly reduced *M.javanica* infections and reproduction on tomato. Various workers studied the effect of AM fungi in controlling root-knot nematodes on tomato (Singh *et al.,* 1990).

Heald *et al.* (1989) showed that *M.incognita* suppressed the growth of non-mycorrhizal *Cucumis melo* plant by 84% as compared to 21% in AM inoculated plants at 50mg/g phosphorus. A similar trend was observed in soil with 100 mg/g phosphorus. Smith (1987, 1988) compared mycorrhizal and phosphorus-fertilized non-mycorrhinzal plants of similar size and found that the latter are more susceptible to nematode attack indicating the likely involvement of factors other than phosphorus nutrition in the interaction. On the other hand, mycorrhizal inoculations of tamarillo *(Cyphomandra betacea)* against *M.incognita* could not be duplicated by adding phosphorus and fertilizer was not therefore, merely due to improved phosphorus nutritions of the host (Cooper and Grandison, 1987) Carling *et al.* (1989) found that egg production by *M.incognita* on AM inoculated plants of soybean was suppressed at the lowest phosphorus rate as well as by increased phosphorus fertilization indicating the induced resistance is possibly due to improved phosphorus nutrition in the host. Krishna Prasad (1991) reported that the percentage of root-knot nematode *M.incognita* infestations in tobacco seedling was 67.5% at 50 days and 95% at 75 days after sowing in non-mycorrhizal plants and 48% - 52% at 50 days and 73% at 75 days after sowing in mycorrhiza (*Glomus fasciculatum)* inoculated plots. The number of galls, egg masses per plant and eggs per egg mass of infested plants was reduced by 61-89% as a result of AM inoculation. Transplanting of mycorrhizal tobacco seedling into root-knot nematode infested soil showed improved yield compared to non-mycorrhizal plants. Carling *et al.* (1996) showed that AM fungi made peanut plant more tolerant to the nematode and offset the reduction in growth caused by *M.arenaria* at the two lower phosphorus levels.

The ability of mycorrhizal plants to grow well despite infection by nematodes is generally considered to be the principal affect of AM fungi on the interaction of host plants with parasitic nematodes (Hussey and Roncadori, 1982). The time of AM inoculations, whether before, simultaneously or after inoculations with the pathogen or nematode, greatly affects the efficacy of AM fungi in controlling nematodes. Preinoculations of AM fungi, suppressed nematode reproduction and development in roots to a greater degree when compared to plants inoculated simultansously with both the organisms (Suresh and Bagyaraj, 1984; Jain and Sethi, 1987; Taha and Abdel-Kader, 1990). Mycorrhizal infections alter either the reproduction capacity of the nematode or the suitability of the plant as a host, especially in the roots pre-inoculated with AM fungus (Cooper and Grandison, 1986; Sundrababu and Sankaranarayan, 1995). Few studies have reported significantly fewer and small size galls/root system in mycorrhizal roots (Bagyaraj *et al.,* 1979; Kellam and Schenck, 1980; Al-Raddad, 1995). The development and reproduction of nematodes is inhibited in the mycorrhizal roots as compared to non-mycorrhizal roots. There are reports where AM fungi increased host tolerance or susceptibility in various crops (Thomson-Cason *et al.,* 1983; MacGudwin *et al.,* 1985; Carling *et al.,* 1996). A non-specific defence respond induced by AM fungi (Valopin *et al.,* 1994; Azcon-Aguilar and Barrea, 1996) might prove to be additional factors limiting *Meloidogyne* population development in the mycorrhizal plants.

Biocontrol efficiency of AM fungi is variable. The obvious effects are due to multiple factors operating together like competition with the nematode for resources, enhancement of plant nutrition, alleviation of physical stresses, changes in biochemical compounds related with plant resistance response and changes in antagonists and deleterious microbial populations in the mycorhizosphere. Biochemical changes include stimulation of the phenyl propanoid changes in levels of aliphatic polyamines, synthesis of proteins, activatation of defence related genes enhancement of certain hydrolase activities, chitinases and glucanases (Morandi, 1996).

Various soil amendments like green manure, organic manure and oil cakes have positive effect on the development and efficiency of AM fungi (St. John *et al.,* 1983). Amendment of botanicals in the nursery beds increased the multiplication of AM fungi providing tomato

seedlings with high colonisation of mycorrhizae which in turn could protect the crop from these nematodes to the maximum extent in the main field resulting in increased yields. These treatments have proved as effective as chemical treatment and in some cases better than chemical treatment (Reddy and Nagesh, 1998). Inoculation of endomycorrhizae - *Glomus mosseae / G.fasciculatum* in nursery beds and subsequent application of 5% aqueous extracts of neem cake/castor cake/neem leaf/calatropis leaf in the nursery beds resulted in the effective management of root-knot nematodes and yielded healthy brinjal seedings which could withstand the attack of nematodes after transplanting in the main field (Mukerji *et al.,* 2000).

7.   Integrated Nematode Management

Integrated nematode management (INM) involves simultaneously or sequentially the use of several methods of control like, including predators and parasites, genetic resistance of hosts, soil amendment, cultural practices and environmental modification. Predators and parasites have already been discussed earlier.

7.1. Genetic resistance

Resistance of the plants can be effective against several species or only specific biotypes of a species. Several dominant or semidominant resistance genes have been identified and mapped to chromosomal location or linkage group (Williamson and Hussey, 1996). A single recessive gene (*mj*) for resistance to the root-knot nematode *Meloidogyne javanica* in the cucumber *i.e. Cucumis sativus* var. *hardwickii* line (J90430) has been idendified. Brenner *et al.* (1998) characterised a tomato gene (*Le Mir*) which encoded a protein with high similarity to miraculin, a flavorless protein. The gene has a sequence similar to the soyabean trypsin inhibitor family. Rapid induction of expression upon nematode infection localised to root tips. Potential anti-nematode genes include those encoding proteinase inhibitors, collagenase or toxins. It was found that when cowpea trypsin inhibitor (CPTI) is expressed in transgenic potatoes, it reduces the

fecundity of root-knot nematodes *Meloidogyne* spp. and shifts the ex-ratio of the *Globodera pallida* to favour the production of males. Various binding sites (Carbohydrate residues) were found on the surface of phytonematodes. Marban-Mendoza *et al* (1987) found that concanavalin applied as a soil drench reduced the galling caused by *M. incognita* in tomato roots. The potential of GNA (Snow drop lectin) as a nematode resistance has been tested in a series of trials, using transgenic oil seed rape and potatoes. Plant can be transformed with genes encoding monoclonal antibodies or single chain antibodies to specify nematode components in an attempt to block the establishment of a feeding site (Baum *et al.,* 1996; Rosso *et al.,* 1996). In tomato a single dominant gene (subsequently referred to as the *Mi* gene) has been widely used in plant breeding efforts and varietal development which confers resistance to all of the economically important species of root-knot nematode including *M.incognita, M.arenaria* and *M.javanica.* In Florida, commercially resistant fresh market varieties climatically and horticulturarly adapted, have become available in the tomato variety 'Sanibel'. Several varieties of potato have effective resistance to corky ringspot (CRS) disease which is transmitted by stubby-root nematodes; these should be planted in fields where CRS is known to occur. These resistant varieties include 'Pungo'; 'Green Mountain', 'Bel Rus', 'Hudson' and 'Superior'. The effect of oil radish cv. Trez and rapeseed cv. Humus as green manure in potato crop was investigated in field plots. Green manure crops were planted after harvest of wheat and incorporated as green manure in Mid October. Potato was planted the following spring. Fall fallow treatments were included as a standard. After green manure incorporation and before planting potato, nematode population densities declined in all pots. Green manure plots had lower *M.chitwoodi* populations than fallow plots and yield of marketable potatoes increased by 106-185% (Al-Rehiayani and Hafez, 1998).

7.2. Soil amendments

Organic soil amendments is the attempt to develop management strategies which promote naturally occuring microorganisms capable of destroying plant parasitic nematodes. Addition of organic matter

to the soil stimulates the activities of microorganisms like bacteria and fungi. Proliferation of microorganism results in increased enzymatic activities of the amended soil and accumulation of specific end products which may be nematicidal (Alam *et al.,* 1979; Badra *et al.,* 1979; Johnson, 1974; Mian and Rodriguez-Kabana, 1982 a,b; Mian *et al.,* 1982; Akhtar and Malik, 2000; Van den Boogert *et al.,* 1994; Chavarria-Carvajal and Rodriguez-Kabana, 1998; Al-Rehiayani and Hafez, 1998; Chen *et al.,* 2000). It has been shown that efficacy of organic amendments against nematodes depends on the chemical and physical properties of the amendment (Rodriguez-Kabana, 1986). The mode of action of organic amendments leading to plant disease control and stimulation of microorganisms is complex and dependent on the nature of the amendments. Nematicidal compounds are released from decomposing organic matter or synthesized by microorganisms involved in the decay process. Microbial by-products include organic acids, hydrogen sulfide, phenols, tannins and nitrogenous compounds (Mian and Rodriguez-Kabana, 1982b). The use of nitrogenous organic matter as soil amendment is a successful strategy for the management of *Meloidogyne* spp. in vegetables and other root knot susceptible crops (Mian and Rodriguez-Kabana, 1982a, 1990). Activities of several soil enzymes have been used as indices of microbial activity in ecological studies (Chavarria-Carvajal, 1997; Kokalis-Burelle and Rodriguez-Kabana, 1994).

7.2.1 Organic & Green Manure

Linkage between organic amendment and reduction of root-knot nematodes by nematophagous fungi in response to organic matter (chopped pine apple plants; sugarcane and a coarse grass) was first suggested by Linford (1937) and Linford *et al.* (1938). Stockoli (1952) reported the effects of a wide variety of organic additives on total soil nematode numbers but presented little information on influences on specific types of nematodes. Lear (1959) obtained significant reductions of three phytophagous nematode species with large quantities of castor pomace added to infested soil.

Devi and Gupta (1995) tested utility of four plants *viz*, mung, cowpea, sunhemp and dhaincha (*Sesbania aculeata)* as green manure on pigeon pea plants infected with *Heterodera cajani* where sunhemp gave best performance. The biofumigation potential of *Brassica* and other members has been investigated by several workers (Sarwar and Kirkegaard, 1998; Sarwar *et al.,* 1998). The glucosinolates present in the tissues on hydrolysis in soil releases compounds like thiocyanates, isothiocyanates, nitrites or oxazoli denethiones which are highly

biocidal to nematodes and other organisms (Brown and Morra, 1997; Rosa *et al.,* 1997). Mojtahedi *et al* (1991) reported suppression of root-knot nematode population with selected *Brasssica* cultivars as green manure. Other crops, velvet bean along with rapesed and supplemental urea were tested to control *M.arearia* and *M.incognita* by Crow *et al.* (1996). Chavarria-Carvajal and Rodriguez-Kabana (1998) evaluated four organic amendments (velvetbean, kudzu, pine bark and urea N) for the management of the root-knot nematode. Most organic amendments were effective in reducing root galling and root-knot nematodes and increasing populations of non-parasitic nematodes. Catalase and esterase were sharply increased by velvetbean, kudzu and pine bark. Their results suggest that complex modes of action operating in amended soils are responsible for suppression of *M.incognita.* Al-Rehiayani and Hafez (1998) amended the soil with green residues of horsebean, velvet bean, castorbean, sudangrass, rapeseed, corn or oil radish. They effectively reduced *M. chitwoodi* populations (79% to 94%). Sudangrass and sorghum-sudangrass grown as cover crops and green manure crops reduced population of *Meloidogyne* spp. (Mojtahedi *et al.,* 1993).

Various organic amendments such as crop residue, oil seed cakes, hay, poultray manure, coffee grinds are effective in controlling economically important plant-parasitic nematodes. Addition of nitrogenous organic manure to soil have been found to reduce population densities of phytonematodes. Effective amendments are compost and animal manure, oil cakes with low C:N ratios. Incorporation of these materials in soil stimulates the soil microflora leading to release of ammonia through the activity of proteolytic and deaminating enzymes. These amendments typically result in the enhancement of soil urease activity and accumulation of ammonical and nitrate nitrogen (Mian *et al.,* 1982; Mian and Rodriguez-Kabana, 1982).

7.2.2 Chitin

Chitin, a polymer of unbranched chains of (1→4) linked 2 acetamino 2 deoxy – D glucose residues, is the most common polysaccharide in nature whose basic unit is an amino sugar (Muzzarelli, 1977). Chitin is considered a permanent component of egg shells of plant parasitic nematodes (Bird and Mc Clure, 1976; Bird and Self, 1995) and has also been detected in the gelatinous matrix of the root-knot nematode *M. javanica* (Spiegel and Cohn, 1985). During the microbial breakdown of Chitin, several substances are liberated. Characterization of these products has revealed the presence of N-acetyl glucosamine, acetic acid and ammonia. Muzzarelli (1977) postulated a mechanism for the degradation that the polymer is probably hydrolysed to yield N-acetyglucosamine, which is then converted to acetic acid and glucosamine and the ammonia is liberated from the latter compound or one of its derivatives. There is a particular microflora associated with the decompostion of chitin in soil (Godoy *et al.,* 1983; Mian *et al.,* 1982; Rodriguez–Kabana *et al.,* 1984). An association has been established between chitnolytic ability of fungi and their capaicty to destroy nematode eggs (Godoy *et al.,* 1982, 1983).

Chitin amendments to soil are effective for the control of nematodes (Godoy *et al.,* 1983, Mankau and Das, 1969: Mian *et al.,* 1982: Rodriguez-Kabana *et al.,* 1984, 1987; Spiegel *et al.,* 1986, 1987, 1988; Mittal *et al.,* 1992, 1995, 1999). A possible amendment is to use nematode components, for example cuticle egg shell, gelatinous matrix *etc*. Addition of these specific compounds to soil would be expected to stimulate development of microbial species capable of degrading similar compounds present in the nematode. Use of chitin offers the advantage of detemining the microbial species that will be present in the amended soil for some time after the application of the amendments. The effect of chitin amendments on nematodes may last for several months. After addition of chitin, sufficient time must be given to develop specialized organisms to levels adequate for effective nematode control. Good control often occurs in the second crop following harvest of the first (Rodriguez-Kabana, 1987). Number of chitinolytic microorganisms especially actinomycetes and bacteria were higher in chitin amended soil compared with the control and they have significantly reduced populations of root-knot nematodes (Rodriguez-Kabana and Morgan Jones, 1987; Speigel *et al.,* 1986 1987). Godoy *et al* (1983) studied the effect of chitin added to soil for the control of *M. arenaria* and its effect on the soil microflora. They found that the addition of chitin to soil reduced nematode populations. Rodriguez-Kabana *et al.* (1984) added crustacean chitin to soil at rates of 0.5-4% (w/w) to control *Heterodera glycines* in the roots of soybean (*Glycine max*) plants. Eight weeks after the amendment, the total number of chitinolytic fungi and actinomycetes in the soil increased. Fungal species isolated from chitin-treated soils are known parasites of eggs of *Globodera, Heterodera* and *Meloidogyne* spp. Culbreath *et al.* (1985) demonstrated that the addition of lignohemicellulosic materials to soil amended with chitin can increase the effectiveness of chitin against nematodes and avoid some of the deleterious effects of chitin when applied at high levels (1.0% w/w).

Various experiments suggest that chitin or other appropriate materials serve as the substrate for the selective development of the biocontrol agent in soil. An increase in number and activity of a specialized mycoflora is likely responsible for the extended control of plant parasitic nematodes observed in soil amended with chitin.

8.   Nematode Control in Some Vegetable Crops

8.1 Potato

The most important pests of potato are root-knot nematode (principally *Meloidogyne incognita),* potato cyst nematode (*Globodera rostochiensis)*, sting nematode *(Belonolaimus longicaudatus)* and stubby-root nematodes.

Root-knot nematodes infest roots and tubers causing root injury that severely reduces yields and may accelerate the "early dying disease". Root-knot nematode galling of tubers is a serious quality defect. Sting nematodes cause pruning, stunting and necrosis of roots, depressing yields significantly. Thigh populations of sting nematodes are often associated with severely mishappen, scruffy and abnormally resulted tubers. Stubby root nematodes apparently cause little direct damage to potatoes. Several other nematodes including spiral lesion, awl, stunt and sheath are associated with reduced yields or quality of potatoes. Nematode species and population levels, incidence of soil borne diseases, soil moisture at field preparation time and the intended market for the potatoes can all affect the choice of nematode control measures. A combination of as many different kinds of control measures as feasible should be used since none provides perfect protection for the crop.

Plant symptoms and yield reductions are often directly related to preplant infestation levels in soil and to other environmental stresses imposed upon the plant during crop growth. As infestation levels increase, so then does the amount of damage and yield loss. *Paecilomyces lilacinus* infecting eggs of *Meloidogyne incognita-acrita* was discovered in Peru and when placed in soil containing nematode-infected plants, 70-90% of the egg masses of *M.incognita-acrita* and *Globodera pallida* were infected after 1 month (Franco *et al.,* 1981; Jatala *et al.,* 1979). In field trials against *M.incognita* parasitizing potato, 86% of the egg masses were infected and 55% of the eggs in those masses were destroyed (Jatala *et al.,* 1980).

Survey conducted on potato in the Maritime provinces have indicated that *Pratylenchus* spp. are the dominant plant parasitic nematodes in roots and soils (Kimpinski, 1979, 1987; Kimpinski and Smith, 1988, Kimpinski and Thompson, 1990). *Pratylenchus penetrans* has reduced yields significantly in potato on Prince Edward Island (Kimpinski, 1982; 1986) and in the United States. The effects of potato cyst nematodes (*Globodera rstochiensis* and *G.pallida)* on the growth of early and later maturing potato cultivars, with or without the *H1* resistance gene were compared in pot experiments and the effects of introducing *Verticillium dahliae* into the system were studied

(Evans, 1987). Early maturing cultivars were less tolerant of nematode attack than late maturing ones, and these with the H1 resistance gene were more tolerant to *G.rostochiensis* than *G. pallida.*

All potatoes are susceptible to nematodes except for a few cultivars resistant to the golden nematode. Cover crops of winter rapeseed significantly reduced subsequent damage to potatoes by root-knot nematodes. Sudangrass reduced soil nematode populations but suppression may not be of sufficient duration for commercial potato production. Using sesame as a cover crop has been reported to decrease nematodes. Velvis and Kamp (1995) studied the infection of second stage juveniles of potato cyst nematodes (PCN) *G. pallida* by *Hirsutela rhossiliensis.* Soil was collected from five experimental plots which had annual crops of potatoes for atleast nine years. The percentage of fungus infected juveniles ranged from 10 to 60%. After 12 weekly additions of *G.pallida* juvenies, the percentage of infected individuals increased to 45-90%. The fungal infection of juveniles of potato cyst nematodes was also studied in soil samples from 20 fields in a potato growing area in northeastern Netherlands, where potatoes are intensively grown. Infection by *H.rhossiliensis* was observed in 17 of the 20 field populations of free living juveniles with an average infection percentage of 15%. After 12 weekly additions of *G.pallida* juveniles, the average infection percentage increased to 25% and extended to 100% of the samples.

8.2 Tomato

Olthof and Estey (1963) observed reduction in root-knot of tomato caused by *Meloidogyne hapla* in sterilised soil amended with dextrose and ammonium nitrate. Mankau (1961) measured reduction in root-knot damage in tomato and okra by *M. hapla* using *Arthrobotrys dactyloides* and *Dactylella elliposospora.* Economic control of *Meloidogyne* spp. By *Dactylella oviparasitica* did not occur on tomato plants (Stirling *et al.,* 1979). Cayrol and Frankowski (1979) developed an isolate of *Arthrobotrys* against root-knot nematodes in tomato. They grew *A. irregularis* commercially (Royal 350) and tried it in fields of several vegetable growers, at a rate of 140g/m2. This rate

resulted in good protection of tomatoes against *Meloidogyne* and satisfactory colonisation of the soil by the fungus. Mankau and Wu (1985) studied the effect of six isolates of nematode-trapping fungus *Monacrosporium ellipsosporum* on *M. incognita* population in field. The most aggressive isolate selected from *in vitro* tests, was tested in greenhouse and field trials for protection of tomato from *M.incognita*. The fungus effectively controled plant damage at 5g and 10g levels and reduced galling by 42% and 49% respectively in pot trials. In field tests, reduction of *M. incognita* and improved plant growth was directly correlated with the amount of fungus used. Noe and Sasser (1995) noted significant control of *M.incognita* infected with *Paecilomyces lilacinus* in tomato and okra. Cabanillas *et al* (1988) observed absence of root galling and giant cell formation in tomato roots incolculated with nematode eggs infected with *P.lilacinus*. Ekanayake and Jayasundara (1994) studied the effect of two nematophagous fungi *P.lilacinus* and *Beauveria bassiana* for use as biocontrol agent against *Meloidogyne incognita* on tomato in Sri Lanka and compared with carbofuran. Carbofuran and *P.lilacinus* controlled the root-knot nematode and increased the growth of plants. Johnson (1959) reported the reduction of severity of root-knot disease on tomatoes by 11 crop residues incorporated into soil.

The efficacy of *P.lilacinus* alone and in combination with chitin in controlling *M. incognita* in tomato plants was studied. Growth parameters were measured after 30, 60 and 90 days. The growth was assessed in terms of shoot/root length fresh weight and dry weight and number of galls per gram fresh root weight. The results show that *P.lilacinus* and chitin treated plants showed improved plant growth in terms of height. The galling was lowest in this treatment. In this case either *P.lilacinus* might have increased the effectiveness of chitin against the nematodes or *vice-versa* (Mittal *et al.,* 1992;1995;1999). Khan and Saxena (1997) found successful control of *Meloidogyne javanica* in tomato by using organic materials and *P.lilacinus*.

Spiegel *et al* (1986) effectively controlled *M.javanica* infestation in tomato and bean seedlings using clandosan prepared from crustaclan chitin. Rodriguez- Kabana *et al* (1984) studied the long term effects of chitin amendments on the root-knot nematode *M. arenaria*. The

incidence of galls caused by the nematode in roots of squash that had been planted a short time after amending the soil with chitin was not affected by the amendments. However, when tomatoes were planted after the squash, the amendments were very effective in reducing the numbers of root galls and preventing production of juvenile nematodes in the roots.

Rao *et al* (1998) evaluated the effect of *Verticillium chlamyd-osporium* and *Pasteuria penetrans* either singly or in combination for the management of *M. incognita* infecting tomato. In the nursery, *V.chlamydosporium* alone or in combination with *P.penetrans* was effective in increasing plant growth parameters of tomato seedlings. In the field, integration of both the bioagents was most effective in reducing root galling, number of eggs per egg mass, nematode population in roots and soil, and in increasing root colonisation and egg parasitilization with *V. chlamydosporium,* infection of *M.incognita* females with *P.penetrans* and tomato fruit yield. Reddy *et al* (1997) evaluated the effect of neem cake at $1kg/m^2$, *P. penetrans* at $28x10^7$ spores/m^2 and *Paecilomyces lilacinus* at 20g inoculum/m^2 either singly or in combination for the management of *M.incognila* infecting tomato under nursery and field conditions. Integration of all the 3 components gave maximum increase in plant growth parameters and the number of seedlings per bed. Parasitism of *M.incognita* females was maximum when neem cake was integrated with *P penetrans* while egg parasitism was highest when neem cake was integrated with *P.lilacinus.* When Stirling and Smith (1998) applied *V. chlamydosporium* in granulated form in the fields of tomato, population density of the fungus increased to about 10^4 cfu/g soil after 7-14 weeks. It was found that 37 and 82% of the first generation egg masses produced by *M.javanica* contained parasitized eggs.

8.3 Okra

An integral management of *Meloidogyne incognita* infecting okra using neem or karanj oil cake at 0.5 ton/ha along with carbofuran at 1 kg. ai per ha was achieved. The above treatments gave maximum reduction in root galling with consequent increase in okra fruit yield. Seed treatment of okra with 5% aqueous extracts of neem leaf/neem cake

containing spores of *Paecilomyces lilacinus/Verticillium chlamydosporium* was found to be effective in the management of root-knot nematode under field conditions and these treatments increased okra fruity yield. Spot treatment or inoculation with VAM fungi *Glomus mosseae, G. fasciculatum* and subsequent application of neem cake/castor cake extracts (5%) facilitated the management of root-knot nematode and increased the yield of okra (Reddy and Nagesh, 1998). Qualitative analysis of microflora present in the rhizosphere of okra during all stages of plant growth showed the presence of egg parasitizing and antagoinistic fungi such as *Paecilomyces lilacinus, P.variotii, P.fusisporus, Acremonium* sp*., Gliocladium* sp*. Trichoderma* sp and *Verticillium* sp. (Rawat *et al.,* 1999). The presence of antagonistic and egg parasitizing fungi in the rhisosphere of Pusa Makhmali Cultivar of okra (which is tolerant to root-knot nematode) and their absence in the rhizosphere of New Pusa-4 cultivar of okra (which is highly suspectible to root-knot nematode attack) is probably due to the role played by the root exudation of galled and non-galled roots. In okra, organic amendments such as azolla, water soluble extracts of oil cakes and neem derivatives have been successfully used in controlling root-knot nematodes (Abid *et al.,* 1995).

Rao *et al.* (1997) controlled *Meloidogyne incognita* in okra by combining *P.lilacinus* with castor cake suspension. Seed treatment and soil drenching with this suspension proved as effective as nematicide carbofuran in reducing the final population of root-knot-nematode and increasing fruit yield of okra.

8.4 Cabbage

Spiegel *et al* (1988) grew cabbage plants in soil amended with clandosan prepared from crustacean chitin (0.3% w/w). The plants were maintained in constant temperature tanks set to 15 or 30° C in soils naturaly infested with the cyst nematode *Heterodera schachtii* or inoculated with the root-knot nematode *Meloidogyne javanica* respectively. Clandosan at 30° C induced an increase in the number of fungi and bacteria; at 15° C such an increase was detected only with chitinolytic microorganism. At 30° C the increase in ammonium

concentration and chitinolytic microorganisms control led the *Meloidogyne* juveniles (Mian *et al.,* 1982; Spiegel *et al.,* 1987; Emma, 1985). Bourne *et al* (1996) studied the effect of the host plant on the efficacy of *Verticillium chlamydosporium* as a biological control agent for root-knot nematodes in four experiments. The growth of the fungus in the rhizosphere differed significantly with different plant species Cabbage and Kale supported the most extensive colonization of the fungus.

8.5 Lettuce

The root-knot nematode *Meloidogyne hapla* is a major pathogen that causes significant losses in lettuce (*Lactuca sativa)*. Severely infected lettuce plants often fail to produce marketable heads and thus, are often not harvested. Crop rotation is of limited value for control for root-knot nematodes in organic soil. Chen *et al* (2000) controlled *Meloidogyne hapla* infecting lettuce by applying spore suspensions of *Bacillus thuringiensis, Paecilomyces marquandii* and *Streptomyces costaricanus* to the soil. Chitin, wheat mash, or brewery compost were incorporated into unfumigated and methyl bromide-fumigated organic soils. All the bacterial and fungal antagonists applied without a soil amendment, except the *B. thuringiensis + S.costaricanus* treatment, reduced root galling and increased lettuce head weight in the unifumigated organic soil, but not in the fumigated soil. All three organic amendments were also effective against *M.hapla* and reduced root galling in fumigated and unfumigated soils. Wheat mash amendment increased lettuce head weight in the unfumigated soil. Similar reductions in *M.hapla* populations and its damage to lettuce were observed by amending soil in large field microplots with *Hirsutella rhossiliensis + Paecilomyces marquandii; H.rhossiliensis + Verticillium chlamydosporium; B.thuringiensis + S.costaricanus* and *B.thuringiensis + S.costaricanus + P.marquandii.*

Certain amendments may contribute to the establishment of a healthier rhizosphere environment for the growth of lettuce plants for example wheat mash amendment increased the number of soil nematodes (Chen *et al.,* 1996). The suppression of *M.hapla* observed in these trials may be the result of an increase in soil microbial activities.

9.   PCR-Based Studies

Molecular approaches are being added to microscopical, physiological and biochemical techniques in order to have knowledge of the molecular basis of the interactions between nematodes and their microbial natural enemies. Host recognition and infection processes of nematophagous fungi have been well studies in *Arthrobotrys oligospora* (Nordbring Hertz, 1988; Tunlid *et al.,* 1992; Lopez-Llorca *et al.,* 2002) and *Verticillium chlamydosporium* (Kerry, 2000; Bordallo *et al.,* 2002). Different fungal isolates are known to vary in their biocontrol efficacy and both the plant and nematode host are implicated in this interaction (Bourne *et al.,* 1994; De Leij *et al.,* 1993)*.* The importance of such variation for the regulation of nematode populations is unclear. Different *V. chlamydosporium* isolates grown in pure culture can be discriminated using PCR-based DNA fingerprinting (Arora *et al.,* 1996). PCR-based methods to detect mycorrhizal and root pathogenic fungi have been reported (Gardes *et al.,* 1991; Lovic *et al.,* 1995). These have relied on primers designed to recognize specific sequence within the mitochondrial genome or the transcribed (ITS) and non-transcribed (IGS) spacers within the ribosonal RNA genes (White *et al.,* 1990).

Hirsch *et al.* (2000) developed PCR-based methods to detect *V.chlamydosporium* on infected plant root. Arbitrary ERIC primers and those based on rRNA genes, to identify fungi grown in pure culture, were unsuitable for DNA extracted from nematode infested roots, because of interference by plant and nematode DNA. A novel method utilizing specific primers designed from an amplified and cloned fragment of the *V.chlamydosporium* P.tubulin gene was developed. Although it could not discriminate between different isolates of *V.chlamydosporium,* one primer set could identify the fungus on tomato roots infested with root-knot nematodes. The *V.chlamydosporium* P. tubulin sequence data showed close homology to sequence from plant endophytic *Acremonium* and *Epichloe* species and the saprotrophic *Trichoderma viride.*

Hirsch *et al.* (2001) compared several methods for studying *V.chlamydosporium* in soil and root environment. These included a semi selective medium for the fungus, PCR primers specific for the

fungal P. tubulin gene, and monoclonal antibodies. In addition to providing a target for species-specific primers, the P. tubelin gene was implicated in resistance to the fungicides used in the semi selective medium, and the genetic basis for this was investigated. Culture and PCR-based methods were used to screen for the presence of the fungus in field soils known to have been suppressive to cereal cyst nematode and that contained *V.chlamydosporium* populations. Monoclonal antibodies specific for either hyphae or conidia of the fungus were obtained and their application as a tool for visualising the infection process on the root was explored. A competitive PCR (cPCR) assay was developed to quantify *V.chlamydosporium* in soil (Mauchline *et al.,* 2002). Ar-irradiated soil was seeded with different number of chlamydospores from *V. chlamydosporium* isolate 10 and samples were obtained at time intervals of upto 8 weeks. Samples were analysed by cPCR and by plating onto a semiselective medium. The results suggested that saprophytic *V.chlamydosporium* growth did not occur in soil and that the two methods detected different phases of growth. The first stage of growth, DNA replication, was demonstrated by rapid increase in cPCR estimates, and the presumed carrying capacity (PCC) of the soil was reached after only 1 week of incubation. The second stage, an increase in fungal propagules presumably due to cell division, sporulation and hyphal fragmentation was indicated by a less rapid increase in CFU and 3 weeks was required to reach the PCC.

The development of a specific diagnostic tool for biocontrol agents is important for future field studies. Several methods have been developed to investigate variation and the ecology of the nematophagous fungus *V.chlamydosporium* in the root environment. These will provide the basis for a quantitative PCR assay for monitoring growth of the fungus in field soils. Such approaches will provide a complete understanding of the interactions between the fungus, plant and nematode and should allow the exploitation of biocontrol agents more effectively in the future.

10.   Bio Nematicides

Bio nematicides are commercial preparations of fungi which are pathogenic to harmful nematodes. These nematode destroying fungi commonly occur in some soils which are suppressive for nematodes.

The products of these fungi contain enzymes which bring about destruction of the cuticle of nematodes. Some commercial preparations of nematode trapping fungi have been marketed earlier. Cayrol *et al* (1978) developed the use of a commercially prepared isolate of *Arthrobotrys* to protect commercial mushrooms from attack by the destructive mycophagous nematode *Ditylenchus myceliophagus.* They chose *A. robusta* var. *antipolis* for development as a biological control agent (Royal 300). Trials with the fungus, seeded simultaneously with *Agaricus bisporus* into mushroom compost, increased harvests of mushroom by over 28% and reduced nematode populations in the compost by about 40%. Cayrol and Frankowski (1979) developed another isolate of *Arthrobotrys* against root-knot nematode in tomato. They grew *A.irregularis* commercially (Royal 350) and tried in the fields of several vegetable growers at a rate of 140 g/m2. This rate resulted in good protection of tomatoes against *Meloidogyne* and satisfactory colonization of the soil by the fungus.

Large scale production of fungal biomass is generally by liquid fermentation (Powell, 1993). Fungal biomass can also be produced by solid state fermentation for application in the field (Stirling, 1991). Stirting *et al.,* (1998) devised commercial formulations of the nematophagous fungus *Verticillium chlamydosporium* where liquid fermentation system was used for the mass production of fungal biomass. The biomass was mixed with carrier and a binder gum and ingredients were granulated and then dried to produce a biologically active product suitable for application to soil. *Paecilomyces lilacinus* is being commercialized for use against cyst and root-knot nematodes (Kerry, 2000).

Recently 'Nemastin' a bionematicide has been introduced. It is a wetteble powder containing nematophagous fungi *Arthrobotrys conoides, A. oligospora, Paecilomyces lilacinus, P.fumosoroseus* and *Verticillium* c*hlamydosporium*. It can be used for prevention of plant pathogenic nematodes. Target nematodes controlled are *Meloidogyne* spp root-knot nematode), *Heterodera/Globodera* (cyst nematode), *Pratylenchus* spp. (Lesion nematode), *Tylenchulus semipenetrans* (citrus nematode), *Trichodorus* spp. (stubby-root nematode), *Xiphinema* spp. (dagger nematode) and other tylenchid nematodes parasitizing food fibre and ornamental crops. All stages of target nematodes like cysts, larvae and adults are susceptible. 'Nemastin' is

available in 1Kg., 2Kg and 4Kg. in plastic bags. The product can be applied at any stage of crop development like preseeding, vegetative growth and fruiting. 2 Kg. product should be mixed in 200-300 l of water and sprayed over one acre (0.4 ha) of soil. The product should be used at monthly intervals. This product is odorless and produces residue-free food.

'Prosper Nema' is another commercial fungal preparation containing a mix of mycorrhizal spores. Ingredients include selected strains of proprietary fungal spores (1 million CFU per gram) and an inert water soluble carrier. 'Prosper Nema' is acceptable by certification agencies as organic. Prosper liquid products are available in 1,5 and 55 gallon containers as well as in bulk quantity. Prosper dry products are available in 1,5 and 25 pound containers as well as bulk quantity. 'Prosper Nema' is formulated to re-establish the natural population of beneficial fungi by introducing active fungal spores into the upper soil layers. 'Prosper Nema' strongly inhibits the population build-up of nematodes, including the one of the harmful root-knot nematode *Meloidogyne*. Tests involving the egg packages of root-knot nematodes showed that 'Prosper Nema' prevents the hatching of the eggs. The results of several tests have shown that the use of mycorrhizae facilitates a decrease of nematodes in the soil. Best results were realized when small doses of 'Propser Nema' were added approximately once every month. 'Prosper Nema' finds its application in virtually all soil cultivations in agriculture and horticulture.

DiTera (Valent Biosciences Corporation) a new biological nematicide derived from the fermentation of a nematode parasitic isolate of soil hyphomyete fungus *Myrothecium verrucaria,* was introduced in selected markets of North America (Warrior *et al.,* 1999). The product and its formulations have been registered as a microbial nematidicide in several countries. The development of DiTera resulted from its specific nematicidal activity against second-stage juveniles of the root-knot nematode *Meloidogyne incognita* in a target-directed contact assay. Even though most of the initial studies were focussed on *M.incognita,* studies at Abbott Laboratories and elsewhere have confirmed the activity aginst several plant parasitic nematode species

such as citrus (*Tylenchulus semipenetrans),* burrowing (*Radopholus similis*), lesion *(Pratylenchus* spp), ring (*Circonemoides* spp.), sting (*Belonolaimus longicaudatus)* and several other plant parasitic nematodes. Toxicological studies indicated a very favourable acute and non-target toxicity profile and suggest minimal adverse effects on non-target organisms. Field evaluations of DiTera on turf, bananas and field, fruit and vegetable crops indicate a significant reduction in populations of the major nematode pests affecting those crops. Early stage field evaluations in replicated, small pot trials using the liquid Di Tera ES formulation showed significant reduction in cyst nematode population in cauliflower during the early, critical stages of plant growth associated with enhancement of yield in treated plots (Westerdahl *et al.,* 1995). An overall increase in yield comparable to that obtained with the current chemical standards was obtained.

The nematicidal activity of DiTera is due primarily to the presence of many, relatively low-molecular mass, water-soluble compounds, which act synergistically. Fermentation of *M.verrucaria* in selected media results in the optimal production of nematode specific active components.

Twomey *et al.* (2000) studied the effect of the bio-nematicide DiTera, on hatching of *Globodera rostochiensis* and *G.pallida.* Exposing cysts of *G.rostochiensis* and *G.pallida* to 1 and 10% DiTera in distilled water for 5 weeks irreversibly prevented hatch when the cysts were transferred to the natural hatching stimulus potato root diffusate (PRD). Similarly exposing cysts of both species for 5 weeks to 1 and 10% DiTera dissolved in PRD resulted in a significant inhibition of hatch which was irreversible when cysts were transferred to PRD alone. Tests on free eggs indicated that when mixed with PRD, DiTera prevented egg shell permeability change, normally caused by PRD as an initial phase in the hatching process, in a majority of eggs. This indication that DiTera interfered with the specific hatching mechanism of *G.rostochiensis* was reinforced by the lack of hatch inhibition of *Meloidogyne incognita.*

11. Conclusion

The development of biocontrol strategies usually requires much research which has largely been done in laboratories within the public sector. To develop sustainable methods of nematode management, a close collaboration between scientists and growers will be required. There is an urgent need to develop some easy technologies for formulation and mass production of fungi at a commercial scale for field application. Chemical nematicides have proved to be expensive in terms of their effects on the environment, like ozone depletion, carcinogenicity, mutagencity, ground water pollution, air pollution, acute toxicity and harm to non-target organisms.

Sustainable programmes for control of plant parasitic nematodes will require the integration of several management options. Host-plant resistance integrates well with biological control agents and cultural practices such as rotation, green menaures and organic amendments. Plant resistance to plant parasitic nematodes continues to be one of the most economical and environmentally safe methods of managing plant parasitic nematode in vegetable crop production. Understanding the mechanisms of resistance and inheritance of these mechanisms will enable pyramiding of resistance mechanisms to produce vegetable crops with the highest levels and greatest stability of resistance.

The effect of organic amendments on nematodes and on efficacy of biocontrol organisms will aid in development of integrated management schemes. Some of the bacteria and fungi may be used in intergrated nematode management programmes despite some obstacles. The rapidly expanding fields of biotechnology and genetic engineering are likely to have an impact on biological control, although the extent to which new technologies will influence developments is unpredictable. There are increasing number of examiples where some degree of biological control has been achieved through integrated nematode management strategies.

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Section 3
General Themes

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12

Biological Control Mechanisms of Fluorescent *Pseudomonas* Species involved in Control of Root Diseases of Vegetables/ Fruits

V. Anjaiah

ABSTRACT: Root diseases of vegetables and fruits are known to be highly destructive which can cause a significant economic yield loss in those crops. The application of fungicides to control root diseases has been increasingly curtailed by the development of pathogen resistance to many key fungicides, expensive, inconsistence, and negative public perception regarding the safety of pesticides and consequent restrictions on fungicide use. Moreover, chlamydospores and mycelium of certain fungi can survive in the soil for several years. Biological control methods that reduce the population of pathogen in the soil appear to be the most practical method. Therefore, plant disease control has generally been focused on the use of biological control agents. Fluorescent pseudomonads have frequently been considered as effective biological control agents against soil-borne plant pathogens because of their rapid and aggressive colonization of plant roots. It has been shown that the fluorescent pseudomonads exert several mechanisms in suppression of plant root diseases, and that such mechanisms differed among the strains. The aim of this study is to analyze biocontrol mechanisms of fluorescent pseudomonads for the traits or metabolites involved in biocontrol of plant root diseases.

1. Introduction

Diseases are major biological constraints for vegetables and fruit crop production and many pathogens including fungi, bacteria, viruses, mycoplasma and nematodes can infect the crop plants. Among those, fungal diseases are one of the major factors limiting crop production and account for a major proportion of the global economic loss. The major soil-borne fungal diseases are wilt, root-rots and damping-off. They may be distributed worldwide, or of only regional or restricted significance. *Macrophomina* (the cause of dry root rot, charcoal rot

and damping-off of many crop plants) is one of the most destructive plant pathogens prevalent in the tropics and sub tropics, inciting diseases in a wide range of hosts. *Phytophthora* spp. causes root-rots in many crop plants including fruits and vegetable crops. Sclerotial root-rot caused by *Sclerotium* spp. (the cause of collar rot, root rot and damping-off of several economic crops), is a serious seedling disease causing a considerable damage to vegetable, oilseed and pulse crops, and is widely distributed in warm and humid environment. The wilt diseases caused by pathogenic *Fusarium* spp. and *Verticillium* spp., infect numerous crop species and cause a significant yield loss in many vegetable and fruit crops. *Pythium* spp. (the cause of damping-off of many horticultural crops) cause severe diseases in some soils, known as conducive soils. In suppressive soils it appears that the presence of one or more microorganisms antagonistic to the pathogen is responsible for reduction of the severity of the disease symptoms (Weller *et al.,* 2002).

Plant diseases are managed by the application of chemical pesticides, however, they have been proven to cause adverse environmental effects and result in health hazards to humans as well as other organisms including beneficial natural enemies. Soil-borne diseases are difficult to manage by application of chemicals since the causal agents are in the soil where chemicals cannot easily be delivered. Furthermore, relatively few chemicals are available to control such diseases that can be caused by bacteria, fungi, or nematodes. Alternative disease management strategies are needed therefore. Among the several control strategies currently used against diseases attacking crop plants, "biological control" has been gaining attention. Biological control provides an opportunity to augment currently available practices and products for management of plant disease. It provides an alternate means of reducing the incidence of plant diseases without the negative aspects of pesticide application (Papavizas, 1985; Mukerji and Garg, 1988a &b; Cook, 1993). Microorganisms that can grow in the rhizosphere are ideal for use as biocontrol agents, since the rhizosphere provides the front-line defense for roots against pathogens (Chet and Inbar, 1994; Gamard and De Boer, 1995; McCullagh *et al.* 1996).

Disease suppression by biocontrol agents is the sustained manifestation of interactions among the plant, the pathogen, the

biocontrol agent, the microbial community on and around the plant, and the physical environment. Therefore, despite its potential in agricultural applications, biocontrol is one of the most poorly understood areas of plant-microbe interactions (Mukerji *et al.,* 1999). The approach to managing soil-borne diseases is to focus on mechanisms of biological control and factors affecting effectiveness of introduced biocontrol agents, and on microbial interactions that occur in the rhizosphere soil of plants that could be managed as a deterrent to pathogen invasion of roots.

The antagonistic microorganisms (*e.g.* fungi or bacteria) were isolated and applied either to seeds or incorporated into soil with different techniques (Whipps, 1992; Nakata *et al.,* 2000). Many groups of microorganisms have been cited as candidates for use in biological control of vegetables and fruit diseases (Janisiewicz and Korsten, 2002). Antibiosis, competition, induction of host resistance, and predation are some of the mechanisms on which the biological control agents are based. Biologicals in the products are bacterial species like *Agrobacterium radiobacter*, *Bacillus* spp., *Burkholderia cepacia*, *Pseudomonas* spp., *Serratia*, and *Streptomyces* spp; while, other fungal species like *Ampelomyces quisqualis*, *Candida oleophila*, several species of yeasts, *Coniothyrium minitans*, *Myrothecium verrucaria*, and *Trichoderma* and *Gliocladium* have been used to control diseases of vegetables and fruits (Janisiewicz and Korsten, 2002; Utkhede and Smith, 1992; Guinebretiere *et al.,* 1992). Studies in the recent years have pointed out that fluorescent pseudomonads may have considerable potential as biological control agents in control of disease caused by soil borne pathogens. Strains of fluorescent *Pseudomonas* spp. are found in large numbers in association with plant roots; they are nutritionally versatile, have relatively rapid growth rates, and frequently produce antibiotics, siderophore and hydrogen cyanide that are inhibitory *in vitro* against fungal root pathogens. We are identifying sources of variation in biological control and devising ways in which biocontrol can be more consistently successful. We are also investigating the nature and role of microbial communities and characterizing functional groups of microbes relative to their potential activities that could affect plant growth and health. In this text we have discussed the biological control mechanisms of fluorescent pseudomonads involved in control of plant diseases.

2. Fluorescent pseudomonads

Fluorescent pseudomonads are ubiquitous soil microorganisms and common inhabitants of the rhizosphere. These are prominent members of the microflora in the rhizosphere of many crop plants and one of the first to colonize young roots. Certain strains suppress plant diseases by protecting seeds or roots from infection by soilborne fungal and bacterial plant pathogens. Currently, *Pseudomonas* spp. are receiving much attention as biocontrol agents. It has emerged as the largest and potentially most promising group of plant growth-promoting rhizobacteria (PGPR) involved in the biocontrol of plant diseases (Dowling and O'Gara, 1994; O'Sullivan and O'Gara, 1992; Weller, 1988; Davison, 1988; Walsh *et al.,* 2001).

Burr *et al.* (1978) and Kloepper *et al.* (1980a) reported significant increases of potato yields following treatment of tubers with specific strains of *P. fluorescens* and *P. putida*. In summarizing results from field tests, Schroth and Hancock (1982) reported that the fluorescent pseudomonads increased the yield of potato 5-33%, and root weight of radish 60-144%. Colver and Mount (1984) demonstrated that bacterization of potatoes with *P. putudia* reduced greatly post harvest soft-rot disease caused *Erwinia* spp. Biological suppression of potato ring rot by fluorescent pseudomonas was observed in greenhouse and field trials (De la Cruz *et al.* 1992; Gamard & De Boer, 1995). In other field trials, with potato seed pieces of four cultivars, strains of *P. putida* and *P. fluorescens* have been shown to increase dry shoot and root weight and significant yield increase in two of three field trials (Howie and Echandi, 1983). Similarly, treatment of potato seed tubers with fluorescent pseudomonads increased tuber yields by 70% compared to the untreated controls (Geels and Schippers, 1983). Van Peer and Schippers (1988) documented increases in root and shoot fresh weight for tomato, cucumber, lettuce, and potato as a result of bacterization with *Pseudomonas* strains. Protection of pseudomonads against Pythium damping-off in cucumber seedlings has been demonstrated (Sugimoto *et al.,* 1990; McCullagh *et al.,* 1996). The growth and yield promotion of cucumber plants was observed by inoculation of rhizobacteria (Utkhede *et al.*, 1999). De Boer *et al.* (1999) showed the enhanced suppression of Fusarium wilt of radish

by using combination of fluorescent *Pseudomonas* spp. Similarly, biological control of Pythium damping-off and Aphanomyces root rot of peas by application of *P. cepacia* or *P. fluorescens* to seed was also reported (Parke *et al.,* 1991). Co-inoculation of beans with *R. phaseoli* and *P. putida* R 105 in field had no effects on plant biomass however; the nitrogen fixation is increased significantly in these plants (De Freitas *et al.,* 1993). Marschner *et al.* (1997) investigated the root exudation and physiological status of root-colonizing fluorescent pseudomonas in mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum* L.) and reported that Mycorrhizal infection has little effect on the physiological status of bacteria due to decrease root exudation for first 6 days.

Fluorescent *Pseudomonas* spp. have also been implicated in the control of Phytophthora root rot of soybean (Lifshitz *et al.,* 1986), collar rot of peanut by *Aspergillus niger* (Dileep Kumar *et al.,* 1999), root rot of peanut by *Rhizoctonia solani* (Savithiry and Gnanamanickam, 1987), potato seed decay due to *Erwinia carotovora* (Xu and Gross, 1986), several wilt diseases due to *Fusarium* spp. (Scher and Baker, 1982; Sneh *et al.,* 1984; Leeman *et al.,* 1996; De Boer *et al.,* 1999), and fungal diseases of orange and lemon citrus roots (Gardner *et al.,* 1984) and ornamental plants (Yuen and Schroth, 1986) and diseases of vegetable crops (Punja, 1997). It has been shown that a variety of minor pathogens which can have deleterious effects on plant growth, root elongation and seed germination can be controlled by inoculation with plant growth-promoting pseudomonads (Geels and Schippers, 1983). The use of beneficial *Pseudomonas* strains for suppression of phytopathogens and increasing plant yield has, therefore, been amply demonstrated in several experimental systems, including field trials (Colyer and Mount, 1984; Ganesan and Gnanamanickam, 1987; Myatt *et al.,* 1992; Nautiyal, 1997; Dileep Kumar, 1998; Leben *et al.,* 1987).

Pseudomonas strains have been used in many instances in control of replant disease on apple seedlings (Biro *et al.,* 1998), green and blue molds of citrus (Bull *et al.,* 1997), green mold on oranges (Huang *et al.,* 1995), Penicillium wound responses of citrus (Huang *et al.,* 1993), apple wounds by *Botrytis cincera* (Mercier and Wilson, 1994), and Fusarium wilt of tomato (Larkin and Fravel, 1998). The use of

beneficial *Pseudomonas* strains has been demonstrated in control several root diseases in vegetable and fruit crops such as Vertcillium wilt in tomato (Sharma and Nowak, 1998), Fusarium wilt in cauliflower caused by *Fusarium moniliforme* (Rajappan and Ramaraj, 1999) and in banana caused by *F. oxysporum* f. sp. *cubense* (Sivamani and Gnanamanickaym, 1988), postharvest decay in pear (Sugar and Spotts, 1999) and crown gall in grapevine and raspberry (Khmel *et al.,* 1998). Microbial antagonists were used for biocontrol of grey mould diseases of fruits including strawberries (Swadling and Jeffries, 1996, 1998); Penicillium rots of citrus (Wilson and Chalutz, 1989); fruit-rot and die-back of chilli (Jeyalakshmi *et al.,* 1998); foot rot of black pepper (Jubina *et al.,* 1998), control of apple scab caused by *Venturia inaequalis* (Kucheryava *et al.,* 1999) and postharvest diseases of peach (Zhou *et al.,* 1999).

The potential for using strains of beneficial *Pseudomonas* spp. and their biocontrol mechanisms in control of soil-borne fungal pathogens and has been demonstrated on many crops (Table 1).

TABLE 1
Some examples of fluorescent pseudomonads involved in
biocontrol of plant diseases.

Mechanism	Antagonistic *Pseudomonas*	Disease (pathogen)	Host plant	References
Competition:				
	Pseudomonas putida	Damping-off (*Pythium ultimum*)	Pea, Soybean	Paultiz, 1991
	Pseudomonas spp.	Fusarium wilt (*Fusairum solani*)	Cucumber	Elad and Baker, 1985
	Pseudomonas spp.	Fusarium wilt (*F. oxysporum* f.sp. *cumuerinum*)	Cucumber	Sneh *et al.,* 1984
	Pseudomonas spp.	Damping-off (*P. aphanidermatum*)	Cucumber	Elad and Chet, 1987
	Pseudomonas spp.	Damping-off (*P. ultimum*)	Chickpea	Kaiser *et al.,* 1989
Siderophores:				
a) Pyoverdin	*P. putida*	Fusarium wilt (*F. oxysporum*)	Radish	Scher and Baker, 1982
	P. putida	Fusarium wilt (*F. oxysporum*)	Cucumber	Park *et al.,* 1988
	P. aeruginosa	Damping-off (*P. splendens*)	Tomato	Buysens *et al.,* 1996
b) Pyochelin	*P.aeruginosa*	Damping-off (*P. splendens*)	Tomato	Buysens *et al.,* 1996

Table 1 cont.

Antibiotics:				
a) Pyoluteorin	*P. fluorescens*	Damping-off (*P. ultimum*)	Cucumber	Maurhofer *et al.,* 1992; Girlands *et al.,* 2001
b) Pyrrolnitrin:				
	P.chlororaphis I-112	Red core disease *Phytophthora fragariae* var. *fragariae*	Straw-berry	Gulati *et al*., 1999
c) Oomycin A	*P. fluorescens*	Damping-off (*P. ultimum*)		Gutterson *et al.,* 1988; Howie and Suslow, 1991.
	P. cepacia (*Burkholderia cepacia*)	Gray mold (*Botrytis cinerea*); Blue mold (*Penicillium expansum*)	Apples and Pears	Janisiewicz *et al.,* 1991.
d) 2,4- Diacetyl-Phloroglucinol	*P. fluorescens*	Soft rot (*Erwinia carotovorai*)	Potato	Cronin *et al.,* 1997
	P.fluorescens CHAO	Damping-off (*Pythium* spp.)	Cucumber	Girlands *et al.,* 2001
	P. fluorescens 113	Damping-off (*P. ultimum*)	Pea	Landa *et al.,* 2002; Naseby *et al.,* 2001
e) Phenazine-1-carboxylic acid	*P. aeruginosa*	Damping-off (*P. splendens*)	Bean	Anjaiah *et al.,* 1998
	P. aeruginosa	Fusarium wilt (*F. oxysporum* f. sp. c*iceris*)	Chickpea	Anjaiah *et al.,* 1998, 2003
f) Phenazine-1-carboxamide	*P.chlororaphis* PCL1391	Foot and root rot (*F. oxysporum* f. sp. *radicis-lycopersici*)	Tomato	Chin-A-Woeng *et al.,* 2000, 2001
g) Pyocyanin	*P. aeruginosa*	Leaf infection *Botrytis cinerea*	Bean	Anjaiah *et al.,*1997
Induced resistance:				
	Pseudomonas spp.	Anthracnose (*Colletotrichum orbiculare*)	Cucumber	Wei *et al.* 1991
	P. fluorescens	Halo blight *P. syringae*	Bean	Alström, 1991
	P. aeruginosa	Leaf infection (*Botrytis cinerea*)	Bean	De Meyer and Höfte, 1997
	P. fluorescens	Pythium root rot	Cucumber	Ongena *et al.,*1999
	P. fluorescens	Fusarium wilt	Radish	Leeman, *et al.,* 1995 a, b
	P. fluorescens WCS417r	Fusarium wilt	Tomato	Duijff *et al.,* 1998
	P. fluorescens WCS365	Foot and root rot (*F. oxysporum* f. sp. *radicis-lycopersici*)	Tomato	Dekkers *et al.,* 2000

3.   Mechanisms of biocontrol of plant diseases by fluorescent pseudomonads

Traits of fluorescent *Pseudomonas* spp. that are involved in the suppression of plant root pathogens have been reviewed (Burr and

Caesar, 1984; O'Sullivan and O'Gara, 1992; Dowling and O'Gara, 1994; Thomashow and Weller, 1996). These groups of bacteria produce a wide variety of secondary metabolites (Leisinger and Margraff, 1979; Budzikiewicz, 1993), some of which have a direct or indirect antimicrobial effect. Many mechanisms are proposed by which the fluorescent pseudomonads inhibit the growth of deleterious microorganisms (Cook *et al.,* 1995). Competition for sites and nutrients between beneficial pseudomonads and soil-borne plant pathogens on roots or in the rhizosphere contributes to biological control. Pseudomonads also antagonize pathogens by producing one or more metabolites that include siderophores, antibiotics, cyanide and lytic enzymes. Pseudomonads can also induce resistance to certain plant pathogens. This induced disease resistance is an active plant defense mechanism that depends on physical or chemical barriers in the host and is activated by root-colonizing *Pseudomonas* bacteria.

3.1. Site and nutrient competition

Competition for nutrients released by roots and seeds and occupation of sites favored for colonization by fluorescent pseudomonads may probably contribute to disease suppression. Nutrient competition varies in different rhizospheres depending on the available sources of carbon, nitrogen, sulfur, phosphate, and micronutrients. The ability of microbial antagonists to take up these nutrients and make them unavailable for plant pathogens can contribute to disease suppression. Paulitz (1991) reported that the biological control of Pythium damping-off by *P. putida* N1R, applied to soybean and pea seeds, was mediated through competition for seed volatiles which may serve as inducers and nutrients for *P. ultimum.* The concept of pre-emptive competitive exclusion can occur when the introduced biocontrol agent attains a large population size and occupies an ecological niche that overlaps or encompasses the niche of the target pathogen, preventing its establishment on the plant surface Nelson and Maloney, 1992). Such mechanism was shown in the biocontrol of frost injury incited by epiphytic ice-nucleating (Ice$^+$) *P. syringae.* Lindow (1987) constructed an Ice$^-$ strain of an Ice$^+$ pathovar and showed that it could compete and pre-empt the introduced wild type on a leaf surface. The

competitive exclusion of deleterious organisms by fluorescent pseudomonads at the plant root may also be a significant suppressive trait of these biocontrol strains.

The competitive exclusion of deleterious rhizosphere organisms is directly linked to an ability to successfully colonize a root surface. The introduced biocontrol agent should grow and persist, or colonize the surface of the plant root to protect the plant (Weller, 1983). Cell surface characteristics influence attachment to roots, which may be necessary for colonization (James *et al.,* 1985; Anderson *et al.,* 1988). Mutants of *Pseudomonas* impaired in their colonization ability have been identified, and analysis of these mutants indicates that prototrophy for amino acids and vitamin B_1, rapid growth rate, utilization of organic acids, and lipopolysaccharide properties contribute to the colonization ability (de Weger *et al.,* 1995). Mutants of *Pseudomonas* biocontrol bacteria lacking flagella were impaired in potato rhizosphere colonization ability in field soil systems) has been demonstrated by Simons *et al.* (1996). Certain mutations that affect accumulation of secondary metabolites also influence colonization of plants in field soil (Mazzola *et al.,* 1992; Pfender *et al.,* 1993; Natsch *et al.,* 1994). Liddell and Parke (1989) showed the enhanced colonization of pea taproots by fluorescent pseudomonad biocontrol agents by water infiltration into soil. The effects of moisture (Howie *et al.,* 1987), temperature (Loper *et al.,* 1985), and pH (Yuen and Schroth, 1986) on successful root colonization have been investigated; also, in few instances, the initial population size (colonization) of the biocontrol agent was shown to be significantly correlated with disease suppression (Paulitz and Baker, 1987; Parke, 1990; Bull *et al.,* 1991).

3.2. Siderophores

Iron is an essential nutrient for most microorganisms, however, it is extremely limiting in an aerobic aqueous environment due to its low solubility. To overcome this, most microorganisms excrete low molecular weight high-affinity iron chelating compounds, called siderophores, which transport ferric iron into the cell. *Pseudomonas* strains produce siderophores (pyoverdins or pseudobactins), consisting of a fluorescent quinoline group covalently linked to a peptide. There

is typically a strain-dependent variation in the structure of the siderophore produced. Pyoverdins are thought to facilitate biocontrol by sequestering iron in the environment of plant roots which may result in severe iron limitation for the surrounding deleterious organisms, and ultimately suppress their growth (Buyer and Leong, 1986; Leong, 1986). The iron starvation conditions may also prevent the germination of fungal spores (Sneh *et al.,* 1984). Kloepper *et al.* (1980b) were the first to demonstrate the importance of siderophore production as a mechanism of biological control. Subsequent genetic evidence provided by a number of groups indicated that the inhibitory properties of certain fluorescent pseudomonads were abolished in siderophore-negative mutants (Magazin *et al.,* 1986; Loper, 1988). *P. putida* strain WCS358 is a unique strain in that it can utilize not only its own iron-siderophore but also the iron-siderophore complexes of a diversity of other strains. More significantly, a Tn*5*-induced siderophore-negative mutant of strain WCS358 lost the ability to promote the growth of potato (Bakker *et al.,* 1986). Siderophores have been shown to be involved in the suppression of formae speciales of *Fusarium oxysporum* (Elad and Baker, 1985), *G. graminis* var. *tritici* (Kloepper *et al.,* 1980b), *F. oxysporum* f. sp. *dianthi* (Lemanceau *et al.,* 1993), and *Pythium* spp. (Becker and Cook, 1988). Several reviews have summarized the evidence supporting a role for siderophores in biocontrol (Leong, 1986; Loper and Buyer, 1991). Analyses of mutants lacking the ability to produce siderophores suggest that they contribute the suppression of certain fungal and oomycete diseases (Duijff *et al.,* 1994). On the contrary, other studies have shown that siderophore production appears to have little or no role in disease suppression (Hamdan *et al.,* 1991; Paulitz and Loper, 1991). However, recent studies with well defined mutants have substantiated the involvement of pyoverdin siderophores in the control of some diseases (Lemanceau and Alabouvette, 1993; Raaijmakers *et al.,* 1995b; Keel *et al.,* 1989), but they also have shown that iron competition is probably not the only mechanism involved in biocontrol. Furthermore, many fluorescent pseudomonads produce additional siderophores such as pyochelin and salicylic acid (Cox *et al.,* 1981; Meyer *et al.,* 1992; Visca *et al.,* 1993), and studies based on the use of Pvd$^-$ mutants therefore may not accurately indicate the importance of iron

competition in interactions with pathogens (Buysens *et al.* 1996). Pyochelin produced by *P. aeruginosa* 7NSK2 has been implicated in protection of hydroponically grown tomato against postemergence damping-off caused by *Pythium splendens* (Buysens *et al.,* 1996)*,* while salicylic acid, a known inducer of the systemic acquired resistance in plants (Uknes *et al.,* 1993) may contribute to the induction of host plant defenses (De Meyer and Höfte, 1997).

Recently, a biological sensor *pvd-inaZ* was constructed where the ice nucleation reporter gene from *P. syringae* was placed under the control of an iron-regulated promoter of a pyoverdin biosynthetic gene in order to measure the biological availability of iron in the rhizosphere, and it was demonstrated that pyoverdins are produced at the root surface (Loper and Lindow, 1994). The same authors (Loper and Lindow, 1994) showed that Fe(III) is not present at extremely low concentrations on plant surfaces and the bacteria inhabiting the rhizosphere may not experience iron deprivation as severe as that predicted by chemical models for the availability of Fe(III) in soil.

3.3. Mycolytic enzymes

The involvement of mycolytic enzymes produced by *Pseudomonas* strains was first demonstrated in soil arificially infested by *Fusarium oxysporum* f. sp. *cubense* (Mitchell and Alexander, 1961). Lim *et al.* (1991) isolated an antagonistic bacterium, *Pseudomonas stutzeri* YPL-1, that was strongly inhibitory to *F. solani* from ginseng rhizosphere; they showed that the antifungal mechanism of the bacterium may involve a lytic enzyme rather than an antibiotic. *P. stutzeri* YPL-1 produced extracellular chitinase and laminarinase when grown on *F. solani* mycelium or on polymers of chitin and laminarin (Lim *et al.,* 1991). Chitin is a major structural component of many agronomically important pests including fungi, nematodes, and insects. Since plants do not contain chitin in their cell walls, it can be selectively used to protect the plants from chitin-containing pathogenic fungi. Scanning electron microscopy studies revealed the interaction site between *F. solani* hyphae and *P. stutzeri*, and the degradation of *F. solani* mycelium suggested that the extracellular lytic enzymes inhibit the mycelial and germ tube growth rather than spore germination (Lim *et al.,* 1991).

Mycolysis is defined as the loss of protoplasm in fungal structure and enzymatic dissolution of the cell walls. Similarly, Ordentlich *et al.* (1988) showed that chitinase was the key enzyme in the dissolution of hyphae of *Sclerotium rolfsii* by *Serratia marcescens*.

3.4. Antibiotic-mediated suppression

Antibiotic production by some fluorescent *Pseudomonas* spp. is recognized as an important feature in the suppression of plant diseases (Walker *et al.,* 1996). Howell and Stipanovic (1979) showed that pyrrolnitrin produced by a fluorescent pseudomonad was involved in suppression of damping-off, caused by *Rhizoctonia solani,* whereas pyoluteorin produced by another pseudomonad was more effective for the protection from Pythium damping-off (Howell and Stipanovic, 1980). The first antibiotics clearly implicated in biocontrol by fluorescent pseudomonads (*P. fluorescens* strain 2-79 and *P. aureofaciens* strain 30-84) were the phenazine derivatives contributed in control root diseases (Weller and Cook, 1983; Brisbane and Rovira, 1988). *Pseudomonas fluorescens* Hv37a was found to produce an antifungal compound, oomycin A, and to protect from infection caused by *Pythium ultimum* (Gutterson *et al.,* 1986; Howie and Suslow, 1991). *Pseudomonas* strain F113 also produced 2,4-diacetylphloroglucinol which is involved in suppression of Pythium damping-off (Fenton *et al.,* 1992*). P. fluorescens* strain BL915 which produced pyrrolnitrin antibiotics, protected from damping-off caused by *R. solani* (Hill *et al.,* 1994).

Certain *Pseudomonas* strains produce a multitude of inhibitory compounds that contribute to the suppression of various plant diseases. However, not all mechanisms are effective against all pathogens and on all hosts. For example, *P. fluorescens* CHA0 produces 2,4-diacetylphloroglucinol (Phl) and pyoluteorin (Voisard *et al.,* 1989; Keel *et al.,* 1992; Maurhofer *et al.,* 1994; Natsch *et al.,* 1998). The production of pyoluteorin contributes to the suppression of damping-off of cucumber, sweet corn and cress caused *Pythium ultimum* whereas Phl contributed in suppression of *Thielaviopsis basicola* and *G. graminis*. In another system, *P. fluorescens* strain Pf-5 was found to produce pyoluteorin which contributes to the inhibition of *P.*

ultimum, pyrrolnitrin which contributes to the inhibition of *R. solani* and *Pyrenophora tritici-repentis,* and Phl which contributes to the inhibition of all three fungi (Corbell and Loper, 1995). In all the above cases the antibiotic negative mutants had lost the ability to inhibit specific pathogenic fungi indicating that the antibotic synthesis is indeed involved in suppression of phytopathogens. The *in situ* antibiotic production of phenazine-1-carboxylic acid by *P. fluorescens* 2-79 and *P. aureofaciens* 30-84, and Phl by *P. fluorescens* CHA0 was demonstrated (Thomashow *et al.,* 1990; Keel *et al.,* 1992).

3.5. Induced Systemic Resistance in plants

Some biocontrol agents of *Pseudomonas* induce a sustained change in the plant, increasing its tolerance to infection by a pathogen, a phenomenon known as induced resistance. Induced disease resistance is an active plant defense process that depends on physical or chemical barriers in the host, activated by biotic or abiotic inducing agents (Uknes *et al.,* 1993; Zhang *et al.,* 1998). The idea that biocontrol agents might induce resistance in the host was first suggested on the basis of experiments showing that bacterial treatments protected potato tubers from subsequent infection by *Ralstonia* (*Pseudomonas*) *solanacearum* (Kempe and Sequeira, 1983). Heat-killed cells or lipopolysaccharides (LPS) of strain WCS417r applied to roots were also protective, suggesting that LPS could be an inducing signal (Van Peer *et al.,* 1991; Van Peer and Schippers, 1992). Similarly, LPS from bacterial cell surface of *P. fluorescens* WCS374 was as effective as living bacteria to suppress Fusarium wilt of radish (Leeman *et al.,* 1995 a, b). Wei *et al.* (1991) showed that seed treatment with plant-growth promoting rhizobacteria resulted in a reduction of the severity of cucumber anthracnose, a leaf disease caused by *Colletotrichum orbiculare*. In another study, foliar lesions of bean caused by *P. syringae* pv. *phaseolicola* are reduced when the seed is bacterized previously with a plant-benefical strain of *P. fluorescens* (Alström, 1991). In all above cases, the benefical bacterium and the pathogen were spatially separated to eliminiate the possbility that direct antagonism and competition were involved in the interaction.

It was also shown that the biocontrol agent *P. fluorescens* strain CHA0 induced SAR-associated proteins, while confering systemic resistance to a viral pathogen (Maurhofer *et al.,* 1994). Mutants of CHA0 that do not produce the siderophore pyoverdin induced a lower level of resistance, suggesting a novel role for this particular bacterial metabolite, in disease suppression. More recently, De Meyer and Höfte (1997) demonstrated, that not the siderophore pyoverdin, but rather salicylic acid (Métraux *et al.,* 1990), was essential for the induction of resistance in *Phaseolus vulgaris* to *Botrytis cinerea* leaf infection by *P. aeruginosa* 7NSK2, however, they did not exclude a similar role for pyochelin. A phenazine compound, pyocyanin, produced by *P. aeruginosa* SA44 was also involved in the induction of systemic acquired resistance in *Phaseolus vulgaris* to *Botrytis cinerea* leaf infection (Anjaiah *et al.,* 1997).

Zdor and Anderson (1992) observed the induction of mRNAs that encode the pathogenesis-related protein PR1a in bean leaves after root inoculation with some fluorescent pseudomonads. Like classic induced resistance, some fluorescent pseudomonads-induced resistance can provide broad-specturm resistance (Liu *et al.,* 1995) and is correlated with increased amounts of pathogenesis-related (PR) proteins, peroxidases, chitinases and β-1,3-glucanase in plant tissues (Maurhofer *et al.,* 1994; Sayler *et al.,* 1994; Wei *et al.,* 1992; Van peer *et al.,* 1991). Taken together, these observations suggest that certain root-colonizing bacteria may induce systemic resistance in plants (Zehnder *et al.,* 1997; Chen *et al.,* 1998; Cook *et al.,* 1995). Ongena *et al.* (1999) shown the predominant role of induced resistance over siderophores and antibiosis in control of Pythium root rot in cucumber by fluorescent pseudomonads.

It is important to note that, in a given biocontrol agent, more than one mechanism may operate to suppress a pathogen, and that the relative importance of a particular mechanism may vary with the physical or chemical conditions in the rhizosphere.

4.   Antibiotic genes of fluorescent pseudomonads and their regulation

Compounds such as oomycin A, pyoluteorin, 2,4-diacetylphloroglucinol, pyrrolnitrin, and phenazine have been isolated

from pseudomonads involved in suppression of plant diseases. The first report on the cloning of antibiotic genes was published by Gutterson *et al.* (1986). Subsequently, the genes for the biosynthesis of all the above antibiotics involved in disease suppression by fluorescent pseudomonads have been isolated, and their regulation has been studied.

4.1. Oomycin A

P. fluorescens Hv37a, isolated from the root tips of barley, is effective against *P. ultimum in vitro* and protected from Pythium damping-off *in vivo* by the production of oomycin A. This compound was found to be responsible for 70% of the reduction in pre-emergence damping-off and 50% of the reduction in post-emergence damping-off caused by *P. ultimum.* Expression of the oomycin A biosynthetic locus was detected *in situ* after 10 or 24 h of inoculation with a transcriptional fusion to *lacZ* (Howie and Suslow, 1991).

The production of oomycin A by *P. fluorescens* HV37a was induced by glucose (James and Gutterson, 1986). Four transcriptional units, which were involved in antibiotic biosynthesis, were identified, designated as *afuE, afuR, afuAB,* and *afuP.* The *afuAB,* and *afuP* transcripitional units were not linked to the others and were not catabolically induced by glucose, whereas the *afuE* and *afuR* operon transcription is apparently the mechanism whereby glucose regulates antibiotic synthesis. Catabolite induction of the *afuE, afuR* transcription was dependent on the products of the *afuAB, afuP* genes (Gutterson *et al.* 1988). Subsequent mapping and transcriptional analyses of a 15-kb DNA segment identified the biosynthetic operon *afuDEFG*, encompassing about 9 kb and encoding products of 48 kDa, 31 kDa, 37 kDa and 145 kDa respectively (Fig. 1) (Gutterson, 1990).
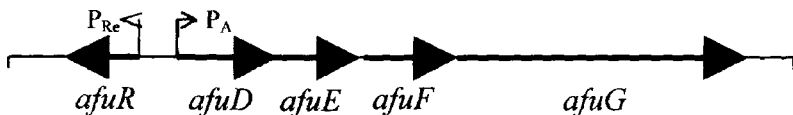
Fig. 1.   Organization of genes involved in oomycin A biosynthesis of *P. fluorescens* HV37a.

The divergently transcribed gene *afuR*, located adjacent to *afuD*, encodes an activator of *afuDEFG* expression; and *afuH*, of unknown function, resides downstream of *afuR* (Gutterson, 1990). Sensing of glucose required expression of the *afuAB* operon, and a mutant in this locus did not produce oomycin A (James and Gutterson, 1986). A second locus, *afuP*, functioned as the response regulator, probably by activating transcription of *afuR* and the *afuDEFG* biosynthetic operon (Gutterson, 1990). The *afuAB* and *afuP* loci have not been characterized, however it is speculated that one or both of them might resemble *lemA* (Corbell and Loper, 1995) or *gacA* (Laville *et al.,* 1992) which focus a two-component system known to be involved in the control of antibiotic synthesis in other fluorescent pseudomonads.

4.2. Pyoluteorin

Pyoluteorin is a chlorinated phenolic polyketide antibiotic. Synthesis *in vitro* is strongly influenced by culture conditions; it was produced by strain Pf-5 on 523 medium, but not on glucose-supplemented nutrient agar (Kraus and Loper, 1992). Pyoluteorin (Plt) is highly inhibitory to *P. ultimum* but not to other pathogens such as *Alternaria* sp., *Fusarium* sp., *R. solani, T. basicola* and *Verticillium dahliae*. Strain Pf-5, produces Plt, increased seedling survival in *P. ultimum*-infested soil; washed cells and culture filtrates were also as effective as whole cultures. Of 6,286 Tn*5* mutants of *P. fluorescens* Pf-5, 13 did not produce detectable Plt and defined five linkage groups; three of these specifically were Plt⁻, whereas the other two exhibited multiple phenotypic defects (Kraus and Loper, 1992; Kraus and Loper, 1995). The Plt⁻ mutants of *P. fluorescens* CHAO are fully effective in protection of cucumber from Pythium damping-off but are less effective in protection of cress (Maurhofer *et al.,* 1994). Thus, pyoluteorin production appears to contribute differentially to suppression of Pythium damping-off diseases of different plant hosts.

Pyoluteorin
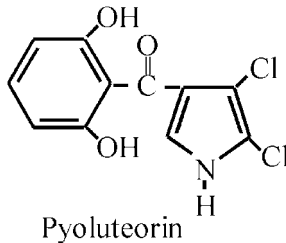
A cosmid clone containing a 21 kb DNA fragment of *P. fluorescens* Pf-5, was identified by cloning the flanking sequences of Tn*5*, and partially restored Plt production in a Plt⁻ deficient mutant of Pf-5 (Kraus and Loper, 1995). A fusion of an ice nucleation reporter gene (*inaZ*) to the Plt production region was constructed to study the expression of the genes required for Plt biosynthesis. This revealed the presence of at least two functional promoters; carbon sources that differentially affected Plt production *in vitro* had parallel effects on the ice nucleation activity, indicating that expression of *inaZ* was controlled from within the Plt region. The expression of ice nucleation activity of the Pf-5 genomic plt::Tn*3*-ice was observed on cotton and cucumber seed planted in field soil. This study demonstrated that the expressions of Pf-5 plt genes by Pf-5 cucumber spermosphere were delayed in comparison with their expression in the cotton spermosphere. Thus, pyoluteorin production appears to contribute differentially to suppression of Pythium damping-off diseases of different plant hosts (Kraus and Loper, 1995). DNA sequence analysis of this region has identified two large genes, *pltB* (7.4 kb) and *pltC* (5.3 kb), whose deduced peptide sequence exhibit characteristics of bacterial Type I polyketide synthases (Fig. 2).
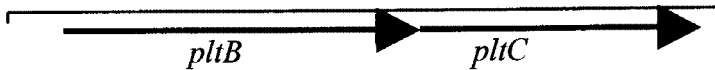
Fig. 2. Organization of genes involved in pyoluteorin biosynthesis (PKS region) of *P. fluorescens* PF-5.

The PltB and PltC proteins are thought to function in assembly of the polyketide moiety of Plt (Nowak-Thompson *et al.,* 1997). Subsequently, the pyoluterin biosynthetic gene cluster of *P. fluorescens* Pf-5 was sequenced and characterized (Nowak-Thompson *et al.,* 1999)

4.3. Pyrrolnitrin

Pyrrolnitrin is a chlorinated phenylpyrrole antibiotic produced from tryptophan by some *Pseudomonas* species. The biosynthetic capability

of pyrrolnitrin is widely distributed among pseudomonads with biocontrol activity against plant pathogens (Howell and Stipanovic, 1979; Lambert *et al.,* 1987; Janisiewicz *et al.,* 1991; Jayaswaral *et al.,* 1991; Hill *et al.,* 1994).
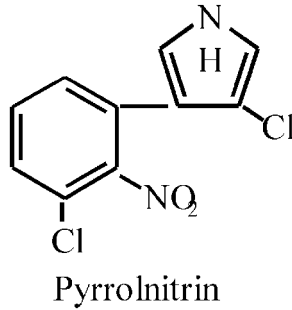
Pyrrolnitrin

P. fluorescens BL915, an isolate from cotton rhizosphere, produces pyrrolnitrin (Prn) as well as hydrogen cyanide, chitinase and gelatinase (Gaffney *et al.,* 1994). A mutant deficient in Prn of this strain lost antagonistic activity *in vitro* and *in vivo* did not suppress damping-off caused by *R. solani* (Hill *et al.,* 1994). A cosmid clone containing pyrrolnitrin biosynthetic genes from BL915 was identified by hybridization with flanking sequences of a Tn5-Prn⁻ mutant from the related strain BL914. Marker-exchange mutagenesis with Tn5 on this cosmid revealed the presence of a 6.2 kb region that contains genes required for the synthesis of pyrrolnitrin (Hammer *et al.,* 1997). This segment contains a cluster of four genes that are required for the production of Prn (Fig. 3).
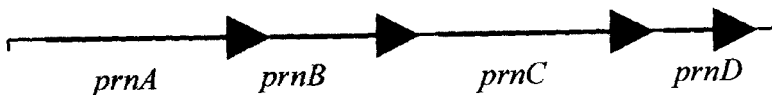
Fig. 3. Organization of genes involved in pyrrolnitrin biosynthesis of *P. fluorescens* BL915.

Each is preceded by a typical ribosome-binding site; two genes, *prnA* and *prnB* appear to be translationally coupled, and the entire cluster appears to be a single transcriptional unit. Deletion mutations in any of the four genes resulted in a Prn-nonproducing phenotype. The predicted protein of 567 amino acids, which is the product of prnC, showed strong similarity to the *chl* gene product, encoding a chlorinating enzyme involved in the synthesis of tetracycline. Three regions of the predicted *prnD* product, a protein of 363 amino acids, resemble highly conserved domains of dioxygenase and demethylase enzymes. Transfer of the gene cluster to *E. coli* resulted in the production of pyrrolnitrin by this organism indicating that the four genes are sufficient for production of Prn (Hammer *et al.,* 1997). The gene cluster involved in biosynthesis of pyrrolnitrin was conserved among the six tested pyrrolnitrin-producing strains (Hammer *et al.,* 1999).

4.4. 2,4-Diacetylphloroglucinol

2,4-Diacetylophloroglucinol (Phl) is a polyketide antibiotic associated with biological control by fluorescent pseudomonads (Bender *et al.* 1999). Many Phl- producing strains have been reported, and in some strains Phl is the major or sole metabolite associated with biocontrol activity *e.g.* in *P. fluorescens* Q2-87, (Vincent *et al.,* 1991), *Pseudomonas* sp. F113 (Shanahan *et al.,* 1992; Fenton *et al.,* 1992), *P. fluorescens* strain 5-2/4 (Alsanius *et al.,* 2002) whereas in other strains it is one of many bioactive compounds *e.g.* in *P. fluorescens* CHA0 (Keel *et al.,* 1992), and *P. fluorescens* Pf-5 (Nowak-Thompson *et al.,* 1994). Phl-producing strains are highly enriched in certain disease-suppressive soils (Raaijmakers *et al.,* 1997), and the *phl* locus is conserved and distributed among plant-associated pseudomonads world-wide (Keel *et al.,* 1996).
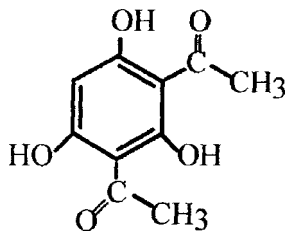
2,4-Diacetylphloroglucinol

P. fluorescens CHA0 produces both monoacetyl- and 2,4-diacetylphloroglucinol, apart from other bioactive compounds (Keel *et al.,* 1992). Phl is the major determinant in the suppression of *G. graminis* by CHA0 (Keel *et al.,* 1992) and also contributed to the control of *T. basicola* (Keel *et al.,* 1990). A Tn*5* mutant, CHA625, deficient in Phl was less inhibitory to *G. graminis* var. *tritici* and *T. basicola in vitro* and also less suppressive *in vivo* than the wild type. Complementation of the mutant with an 11-kb DNA fragment from a genomic library of CHA0 restored Phl production, fungal inhibition, and disease suppression.

Pseudomonas sp. F113 was as effective as a fungicide control treatment in preventing Pythium damping-off *via* the production of Phl (Fenton *et al.,* 1992). Phl presumably is synthesized *via* successive acyl condensation reactions, with acetylation of monoacetylphloroglucinol as the final step (Shanahan *et al.,* 1992). In culture, fructose, sucrose, and mannitol supported high yields of Phl with ammonium ion as the preferred nitrogen source (Shanahan *et al.,* 1992). Phl⁻ Tn*5* mutants of F113 were found to be significantly less suppressive of disease than the respective wild type strain. The Phl mutants were partially restored in their antagonsim upon complementation with wild type DNA fragments. The 6 kb complementing fragment from F113 was expressed in one of eight other non Phl-producing pseudomonads after introduction; the recombinant derived was more protective than its parental strain against *P. ultimum* (Fenton *et al.,* 1992).

P. fluorescens Q2-87, producing Phl and HCN, and is effective against take-all of wheat (Vincent *et al.,* 1991; Pierson and Weller, 1994). Phl⁻ Tn*5* mutants of Q2-87 did not inhibit *G. graminis* var. *tritici in vitro* and did not produce Phl. Two cosmid clones of 25 or 37 kb containing DNA fragments from Q2-87 could complement and restore the antifungal activity and Phl production. Mobilization of these cosmid clones into *Pseudomonas* strains 2-79 and 5097, neither of which produced Phl by itself, conferred the ability to synthesize Phl (Vincent *et al.,* 1991). Further analysis showed that the genes encoding the Phl biosynthesis are contained within a 6.5 kb DNA fragment and are readily expressed in heterologous *Pseudomonas* spp. (Bangera and Thomashow, 1996). DNA sequence analysis of the 6.5 kb fragment

revealed six open reading frames, *phlABCD* which are contained within a single transcriptional unit required for Phl synthesis (Bangera and Thomashow, 1999). This gene cluster is flanked on one side by a divergently transcribed gene, *phlF,* which encodes a 23 kDa protein with similarity with homologues repressors making it a putative regulator of Phl synthesis (Fig. 4) (Delany *et al*. 2000). *phlE* on the other side of the cluster and separately transcribed, encodes a 45.2 kDa protein that resembles integral membrane permeases. The translation of this protein is associated with the excretion of a red pigment in media when Phl is produced, suggesting a role in Phl export (Thomashow and Mavrodi, 1997).
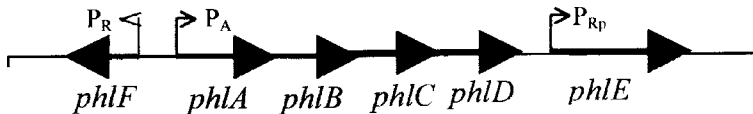
Fig. 4. Organization of genes involved in 2,4-diacetylphloroglucinol biosynthesis of *P. fluorescens* Q2-87.

PhlA, PhlB, PhlC, and PhlD resemble proteins active in acyl transfer and acyl condensation reactions required for fatty acid and polyketide assembly. PhlA is a 37.9 kDa protein with similarity to a β-ketoacyl synthase II from *E. coli*, PhlC is homologous with the thiolase domain at the amino-terminus of mammalian sterol carrier proteins, and PhlD is homologous to plant chalcone and stilbene synthases. Colinear homologues of *phlABC* have been reported to be *acaABC* of *Pyrococcus furiosus*, suggested to play a role in acetoacetyl-CoA synthesis (Bangera and Thomashow, 1996; Thomashow and Mavrodi, 1997). The authors suggest that PhlD is responsible for reactions leading to the formation of monoacetylphloroglucinol, the immediate precursor of Phl, and that the products of *phlABC* together may catalyze the final acetyltransferase reaction (Dowling and O'Gara, 1994) that converts monoacetyl-phloroglucinol to Phl. (Bangera and Thomashow, 1996; Thomashow and Mavrodi, 1997).

4.5. Phenazines

Phenazines are nitrogen-containing heterocyclic molecules with broad spectrum antibiotic properties produced by some bacteria *via* the shikimic acid pathway (Turner and Messenger, 1986). The absorption spectra of phenazines are characteristic with an intensive peak in the range of 250-290 nm and a weaker peak at 350-400 nm. Phenazine-1-carboxylic acid (PCA), produced by the root-colonizing bacteria *P. fluorescens* 2-79 and *P. aureofaciens* 30-84, has a dominant role in the control of the fungus *G. graminis* var. *tritici* (Weller and Cook, 1983; Hamdan *et al.,* 1991). Phenazine-deficient mutants of these strains were greatly reduced in their ability to suppress *G. graminis* compared to the parental strains, whereas genetically complemented mutants were restored both in phenazine production and suppressiveness of *G. graminis* (Pierson and Thomashow, 1992; Thomashow and Weller, 1988). The same mutants are not only defective in pathogen inhibition and disease control, but also are impaired in their ability to effectively compete with the indigenous microflora on plant roots, indicating that phenazines contribute to the ecological competence of the producer strain (Mazzola *et al.,* 1992; Pierson and Pierson, 1996). PCA has been isolated from the rhizosphere colonized by strains 2-79 and 30-84 and its presence correlated with the disease suppression (Thomashow *et al.,* 1990). *P. aureofaciens* 30-84 also produces minor amounts of 2-hydroxyphenazine-1-carboxylic acid and 2-hydroxyphenazine in addition to PCA (Pierson and Thomashow, 1992; Harrison *et al.,* 1993). Phenzine-1-carboxamide, a derivative of phenazines, was shown to be involve in biocontrol of tomato root rot caused by *F. oxysporum* f.sp. *radicis-lycopersici* (Chin *et al.,* 1998) and Fusarium wilt in chickpea and pigeonpea (Anjaiah *et al*., 2003). Pyocynine, another phenazine derivative, was shown to involve in control of *Septoria tritici* (Flaishman *et al.,* 1990).
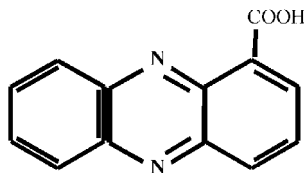
Phenazine-1-carboxylate

The phenazine biosynthetic genes of *P. aureofaciens* have been cloned and sequenced (Pierson *et al.,* 1995). DNA sequence analysis of the *phz* locus predicts seven open reading frames designated *phzI* (Wood and Pierson, 1996), *phzR* (Pierson *et al.,* 1994)*,* and *phzFABCD* (Pierson *et al.,* 1995). Expression of *phzFAB* from strain 30-84 is required for production of PCA in *E. coli*: *phzB,* encodes a 55 kDa protein involved in the biosynthesis of PCA, and *phzC*, encodes a 19 kDa protein involved in the conversion of PCA to 2-hydroxyphenazine-1-carboxylic acid (Fig. 5) (Pierson and Thomashow, 1992; Pierson *et al.,* 1995). The regulatory genes *phzR* and *phzI* linked upstream of the *phz* locus of strains 30-84 and 2-79 are members of the *luxI* / *luxR* gene family (Pierson and Pierson 1996; Fuqua *et al.,* 1994) which control gene expression by a mechanism of quorum sensing *via* the production of homoserine lactones. *phzR* encodes a 27 kDa protein similar to the transcriptional activator *lasR* of *P. aeruginosa* and activates the transcription of phenazine genes in *trans* in response to the product of the *phzI* gene (Pierson *et al.,* 1997).
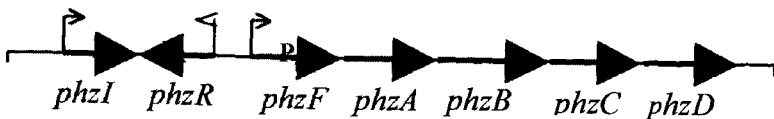
Fig. 5.   Organization of genes involved in phenazine biosynthesis of *P. aureofaciens* 30-84.

A similar locus was also found in strain *P. fluorescens* 2-79. This locus contains the regulatory genes *phzI, phzR*, and the *phzABCDEFG* cluster; with *phzCDEFG* corresponding to *phzFABCD* of strain 30-84 (Fig. 6). The additional genes *phzAB* located upstream of the locus were also co-transcribed with *phzCDEFG* (Thomashow and Mavrodi, 1997).
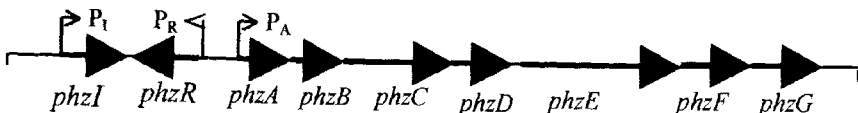
Fig. 6.   Organization of genes involved in phenazine biosynthesis of *P. fluorescens* 2-79.

The biosynthesis of pyocyanin, a blue phenazine pigment, of *P. aeruginosa* PAO1 is not clearly known yet. The *phnAB* genes, encoding for second anthranilate synthase, involved in pyocyanin biosynthesis were cloned and sequenced (Essar *et al.,* 1990). More recently, Thomashow and Mavrodi (1997) have identified the pyocyanin biosynthetic genes designated *pcnCDE* with homology to the *phzCDE* genes of *P. fluorescens* 2-79 from *P. aeruginosa* PAO1 (Fig. 7). However, their function has not yet been demonstrated.
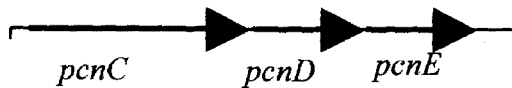
Fig. 7.   Organization of genes involved in pyocyanin biosynthesis of *P. aeruginosa* PA01.

Several authors (Pierson *et al.,* 1995; Thomashow and Mavrodi, 1997; Essar *et al.,* 1990) have showed that the predicted phenazine gene products had similarity with products of shikimate, enterochelin, and tryptophan biosynthetic pathways. PhzF in strain 30-84; PhzC in strain 2-79, and PcnC in strain PAO1 resemble 3-deoxy-D-arabino-heptulosonate-7-phosphate synthases that catalyze the key branch point reaction leading to the shikimate pathway. PhzA in strain 30-84, PhzD in strain 2-79, and PcnD in strain PAO1 resemble 2,3-dihydro-2,3-dihydroxybenzoate synthase (isochorismatase or EntB). PhzB in strain 30-84, PhzE in strain 2-79, and PhnAB and PcnE in strain PAO1 resemble anthranilate synthase or glutamine amidotransferases, respectively . These gene products are likely to modify and condense chorismate to yield phenazine-1-carboxylic acid, which is the first product in phenazine biosynthesis.

The *phz* biosynthetic gene locus of strain 2-79 was expressed for constitutive production of PCA in the plant growth- promoting non phenazine-producing strain *P. putida* WCS385r to study possible adverse effects of genetically modified biocontrol bacteria on the indigenous soil microflora. The WCS358r::*phz* derivative produced significant amounts of PCA, was not impaired in its colonization and it altered composition of the rhizosphere fungal population in microcosm studies (Glandorf *et al.,* 1997).

P. aureofaciens PGS12, isolate from corn roots, showed a broad-spectrum activity *in vitro* against plant pathogenic fungi by the production of PCA, two hydroxyphenazine compounds, pyrrolnitrin and HCN (Georgakopoulos *et al.,* 1994a). An ice nucleation reporter gene fusion to the phenazine biosynthetic locus of strain PGS12, *phz:inaZ*, revealed the effects of availability of various nutrients on the phenazine expression. The expression of the *phz:inaZ* reporter gene was significantly different on germinating seeds of seven different plant species in the soil environment when measured 48 h after planting (Georgakopoulos *et al.,* 1994b). The highest expression level was recorded for wheat seeds (one ice nucleus per 4,000 cells), and the lowest expression level was recorded for cottonseeds (one ice nucleus per 12,000,000 cells). These values indicate that a small proportion of bacteria in a seed population produce phenazine. In addition, the population size and the gene expression levels were lognormally distributed among individual seeds, usually with greater variability in gene expression than in population size. It was suggested that the nutrient level in seed exudates is the primary cause of the differences observed among seeds (Georgakopoulos *et al.,* 1994b).

4.6. Global regulatory mechanisms of control of antibiotic gene expression in fluorescent pseudomonads

Antibiotic biosynthesis is controlled by regulatory systems that coordinate metabolic processes during growth and in response to environmental change. An emerging theme in the fluorescent pseudomonads is that global regulatory elements coordinate the production of secondary metabolites (Whistler *et al.,* 1998).

4.6.1. Two-component sensory transducer and response regulator mediated gene regulation:

In general, two-component systems consist of two proteins; a membrane associated sensor-kinase and a second intracellularly located response-regulator. The sensor responds to a specific environmental signal by auto-phosphorylation at a conserved histidine residue; and the phosphate group is transferred to a conserved aspartate residue on the cytoplasmically located response-regulator, enabling it to bind to regulatory DNA sequences and to modulate the expression of target genes (Wurgler-Murphy and Saito, 1997).

The *lemA* (Hrabak and Willis, 1992) and *gacA* (Laville *et al.,* 1992) homologues function as sensors and activators respectively, and are members of

classic two-component regulatory systems required for the production of antibiotics such as Phl, Prn, Plt and Phz (Thomashow and Mavrodi, 1997; Corbell and Loper, 1995; Gaffney *et al.,* 1994; Laville *et al.,* 1992). Mutants in *lemA* or *gacA* typically exhibit pleiotropic phenotypes with altered colony morphology and do not produce antibiotics including some extracellular enzymes.

The *gacA* locus was originally described in *P. fluorescens* strain CHA0, a biocontrol agent that inhibits fungal pathogens through production of hydrogen cyanide and the antibiotics Phl, Plt and Prn; mutants in *gacA* were found to be deficient in production of all these inhibitory substances (Laville *et al.,* 1992) including the tryptophan side chain oxidase, extracellular protease and phospholipase C (Sacherer *et al.,* 1994). A single gene that restored each of the defective traits was identified from a genomic library of strain CHA0 and was shown to encode a 24 kDa protein with similarity to the response-regulator domains of two component regulatory systems. GacA contains a DNA binding motif and it is believed to serve as the response-regulator of a two component regulatory system (Laville *et al.,* 1992; Duffy and Defago, 2000). Similarly, spontaneous pleiotropic mutants of the *P. fluorescens* strain BL915 which fail to synthesize Prn, chitinase and cyanide were complemented by introduction of an 11 kb *Eco*RI fragment cloned from BL915 that contained a gene coding a protein with similarity to GacA (Gaffney *et al.,* 1994).

The *lemA* gene of the phytopathogen *P. syringae* pv. *syringae* encodes the sensor kinase (Hrabak and Willis, 1992) and is extensively conserved among biocontrol strains of *P. fluorescens* (Pf-5 , BL915 and 2-79). *P. fluorescens* Pf-5 produces Plt, Prn, Phl, and HCN and suppresses the soil-borne fungi *R. solani*, *P. ultimum*, and also ascocarp formation by *Pyrenophora tritici-repentis* (Kraus and Loper, 1992; Corbell and Loper, 1995). A mutant of this strain was affected in the production of all the above compounds, and was designated as ApdA⁻. DNA analysis showed that *apdA* encodes a putative sensor kinase component, which is closely related to *lemA* (Corbell and Loper, 1995). Similarly a pleiotropic mutant of BL915, which had an altered colony morphology and had lost the ability to produce pyrrolnitrin, cyanide and chitinase,was complemented by *lemA* (Lam *et al.,* 1994). In strain 2-79, the mutation in locus *phzP* behaved similarly. The product of this gene is thought to function in conjunction with the product of the *gacA* gene in a two-component signal transduction system (Thomashow *et al.,* 1993). Similarly, in *P. aureofaciens* 30-84, pleiotropic mutants defective in phenazine synthesis were identified which are not complemented by *phzR, phzI* or *phz* biosynthetic genes. However, one set of these mutants was complemented with *lemA* and other set was complemented by *gacA* (Pierson *et al.,* 1997). Spontaneous mutations in *gacA* have been recovered in some strains, a phenomenon that may contribute to the apparent loss of antagonistic activity observed in some strains after laboratory cultivation (Gaffney *et al.,* 1994; Voisard *et al.,* 1994).

4.6.2. N-acyl-homoserine lactone-mediated gene regulation:

Many bacterial species produce acylated homoserine lactone derivatives that accumulate in the milieu and, at a critical concentration, activate certain cellular processes associated with high population density. N-acyl-homoserine lactone-

mediated (N-acyl-HSL) regulation was originally termed 'autoinduction' and more recently 'quorum-sensing' (Fuqua *et al.,* 1994; Pierson III *et al.,* 1998). Signal synthesis depends on the products of genes of *luxI* family, and transcriptional activation is mediated by *luxR* family members (Fuqua *et al.,* 1994). Phz synthesis in *Pseudomonas* strain 30-84 and 2-79 is regulated by the *luxI/luxR* homologues *phzI* and *phzR* (Pierson *et al.,* 1994; Wood and Pierson, 1996; Thomashow and Mavrodi, 1997). *PhzI* is responsible for the production of N-acyl-homoserine lactone; when N-acyl-HSL accumulates to a threshold concentration, it is believed to interact with *PhzR* to bind the specific sequences within the *phz* biosynthetic and *phzI* promoter regions. Binding would activate expression of *phz* biosynthetic genes and would also increase the expression of *phzI.* Amplification of *phzR* in strain 30-84 enhances *Phz* production and fungal inhibition *in vitro* (Pierson *et al.,* 1997).

The expression of extracellular virulence factors and secondary metabolites such as pyocyanin, and cyanide in *P. aeruginosa* PAO1 depends on multiple homoserine lactone regulatory systems homologous to *LuxR* and *LuxI* (Brint and Ohman, 1995; Winson *et al.,* 1995; Latifi *et al.,* 1996; Reimmann *et al.,* 1997) (Fig. 8). The quorum-sensing transcriptional activator LasR and its cognate autoinducer synthase, LasI, are required for expression of a secondary autoregulatory circuit consisting of *RhlR* (VsmR) and RhlI (VsmI), members of the LuxR/LuxI family (Ochsner and Reiser, 1995; Latifi *et al.,* 1996). The inactivation of *lasR* results in the lack of *rhlR* expression (Latifi *et al.,* 1996; Passador *et al.,* 1993).

Recently, a linkage between *GacA* and *LasR* and *RhlR* was demonstrated in *P. aeruginosa PAO1* (Reimmann *et al.,* 1997). Mutational inactivation of *gacA* resulted in delayed and reduced formation of *LasR*, *RhlR* and of N-butyryl-homoserine lactone (N-BHSL; product of the action of the RhlI synthase), which are required for the expression of extracellular virulence factors of the strain. The introduction of additional copies of *gacA* in *trans* resulted in increased production of N-BHSL, *LasR* and *RhlR*; which production of lipase, cyanide, and pyocyanin also increased in parallel with *gacA* gene dosage (Reimmann *et al.,* 1997). Similarly, in *P. aureofaciens* 30-84, the loss of either *lemA* or *gacA* resulted in a loss of N-acyl-homoserine lactones required for the induction of phenazine synthesis in this strain (Pierson *et al.,* 1997).

4.6.3. Sigma factor-mediated gene regulation:
Sigma factors also regulate antibiotic production in fluorescent pseudomonads. Sigma factors are subunits of RNA polymerase that direct transcription and regulate a distinct set of genes, thereby playing a key role in gene regulation in bacteria. Antibiotic synthesis influenced by products of *rpoD* and *rpoS*, which encode the 'housekeeping' and 'stationary phase' sigma factors σ^{70} and σ^S (σ^{38}), respectively.

P. fluorescens CHA0 involved in biocontrol of Pythium damping-off diseases of cress, sweet corn and cucumber by the production of Plt Phl and Prn. A recombinant strain of CHA0 containing a 22 kb CHA0 fragment cloned in a cosmid (pME3090) was more protective than the parental strain CHA0 in suppression of Pythium damping-off in cucumber due to overproduction of Plt
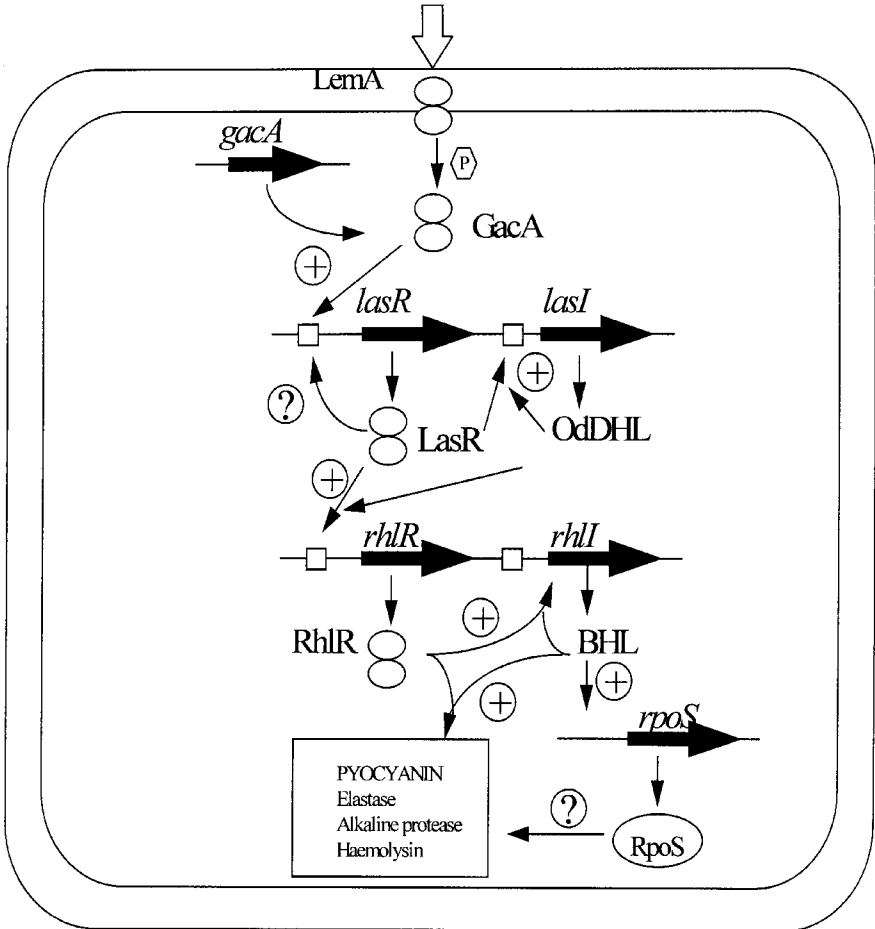
Fig. 8. Hierarchical arrangement of regulatory factors in *P. aeruginosa* PAO1 involved in biosynthesis of pyocyanin and some extracellular enzymes (Pierson *et al.* 1997; Thomashow and Mavrodi, 1997).

(Maurhofer *et al.,* 1992). Subsequently, Schnider *et al.* (1995) showed that both enhanced antibiotic production and improved protection against damping-off of cucumber are due to amplification of a *rpoD* gene encoding the housekeeping sigma factor σ^{70} of *P. fluorescens* of CHA0 (Maurhofer *et al.,* 1995).

Similar effects are observed in an *rpoS* mutant of *P. fluorescens* Pf-5 (Sarniguet *et al.,* 1995). Strain Pf-5 inhibited *Pyrenophora tritici-repentis*, causal agent of tan spot disease of wheat *in vitro*, and suppressed ascocarp development of the fungus *in vivo via* the production of Prn. A Tn5 mutant of Pf-5, JL3985, does not produce Prn and is less suppressive to *Pyrenophora tritici-repentis* on wheat straw than the parental strain. The cosmid pJEL 1884 restored the mutant to Prn production; however, it did not confer Prn production in *P. putida*, a non-

Prn producer (Pfender *et al.,* 1993). Further DNA sequence analysis showed that the mutation was in the *rpoS* gene, which indicates that the production of Prn by this strain requires a functional *rpoS* gene (Sarniguet *et al.,* 1995). RpoS functions in the transition to stationary phase and survival in adverse environment (Hengge-Aronis, 1993), and an *rpoS* mutant is less persistent than the wild type on plant surfaces and residues (Sarniguet *et al.,* 1995; Pfender *et al.,* 1993). Recently, the *rpoS* gene was identified in *P. aureofaciens* 30-84 by using a *rpoS* gene probe from *P. fluorescens* Pf-5. The RpoS of strain 30-84 shared similarity to RpoS of *E. coli* (76%), *P. aeruginosa* PAO1 (92%), and *P. fluorescens* Pf-5 (97%). An *rpoS* mutant of this strain yielded a two fold increase in the level of phenazine production in a rich medium and a reduced phenazine production in minimal medium (Harvey and Pierson, 1997). The ratio of the sigma factor σ^{70} and σ^{S} appears critical in the regulation of various metabolites involved in disease suppression (Schnider *et al.,* 1995).

A linkage between N-acyl-HSL-mediated regulation and the *rpoS* gene regulation was observed in *P. aeruginosa* PAO1 (Latifi *et al.,* 1996). Mutational inactivation of *lasR* resulted in the loss of expression of the plasmid-borne *rpoS:lacZ* fusion. It was hypothesized that the autoinducer N-(3-oxododecanoyl)-homoserine lactone (produced by LasI) enters in competition with N-BHSL for RhlR which could ensure that *rpoS* expression is not induced until sufficient levels of both RhlR and N-BHSL are present within the cell (Pesci *et al.,* 1997). This indicates that there is an hierarchical arrangement of regulatory factors involved in synthesis of extracellular secondary metabolites in *P. aeruginosa* PAO1 (Fig. 8).

5.   Limitations of Biocontrol Agents

Biological control is a viable alternative, but application of biocontrol systems in agriculture is often unsuccessful or variable, largely because of the lack of knowledge of the factors involved in having the level of antagonism against the pathogens be sufficient to prevent the disease or reduce the disease losses to economically sustainable levels. Variable efficacy poses a serious obstacle to the development of biocontrol products by industry and the use of these products by growers. More reliable biocontrol products and processes can be developed if sources of variation are identified and strategies for decreasing variability are implemented. The reliability can be increased by managing the major microbial components that will impact both the biocontrol agents as well as the pathogens. Without this knowledge and application technology, farmers will not realize the benefits of the biological

approach to management of soil-borne diseases, and will suffer losses as a consequence or be forced to continue applying chemical pesticides to the detriment of the environment and people.

Performance of an introduced strain often varies from site to site and year to year. This may be due to many causes, including abiotic factors, poor and inconsistent colonization, and the failure to produce the metabolite(s) at the appropriate time or levels due to fluctuations of environmental conditions. Therefore, it is essential to understand the charcteristics of the biological control agents, including their mechanisms of action, the ecology of the environment for which they are destined, and the aetiology of the diseases involved, for their successful use.

5.1. Variability in root colonization by the introduced bacteria

Variable root colonization undoubtedly is one cause of inconsistent performance of the introduced bacteria (Lemanceau *et al*., 1995). Many factors can contribute for the sub-optimal root colonization by a biocontrol agent given the complex interactions between the host, the pathogen, the bacteria, other microflora, and the soil environment (Weller, 1988; Lugtenberg *et al.,* 1999; Pillay and Nowak, 1997). Bull *et al.* (1991) reported an inverse relationship between the population size of *P. fluorescens* 2-79 and the number of disease plants, indicating that incomplete colonization could reduce the chances for a successful biocontrol. Spatial-temporal colonization patterns of introduced bacterial population are log-normally, rather than normally distributed among roots of different plants (Loper *et al.,* 1985; Mahaffee *et al.,* 1997) and also individual roots of a single plant (Bahme and Schroth, 1987) indicating that population sizes among roots vary by several orders of magnitude meaning that some roots may be completely unprotected. This is probably a main cause for inconsistent control by biocontrol agents.

The nature of the plant and the environmental conditions affect the quantity and composition of root exudates, which in turn determines the population of the rhizosphere microflora. The biological composition of the rhizosphere will dramatically influence root colonization. It was shown that in the rhizoplane and rhizosphere

nutrient availability is the primary determinant of microbial population size rather than space. The introduced bacteria pre-empt the establishment of the normal indigenous population which results in a shift in the composition of the microflora of the rhizosphere (Bowen & Rovira, 1976). The type and quantitiy of root exudates upon which rhizosphere microflora depend are under environmental and genetic control. So, the compostion of the indigenous rhizosphere microflora, as well as the population size of introduced bacteria vary among plant species and varieties of the same crop species (Miller *et al.,* 1989; Kremer *et al.,* 1990; Mukerji, 2002; Anjaiah *et al.,* 2003).

Ecological competence is the ability of an introduced bacterium to compete and survive in nature. Many bacterial traits have been identified as being relevant for efficient root colonization such as the presence of flagella and fimbriae, the presence of cell surface polysaccharides (O-antigenic side chain of LPS), chemotaxis, osmotolerance and utilization of complex carbohydrates. Other traits reported to be relevant for the ability to compete with rhizosphere microorganisms are the production of antibiotics or siderophores and the ability to utilize siderophores of other rhizosphere bacteria. These bacterial traits contribute to the ecological competence in the rhizosphere and loss of any one can reduce the ability of bacteria to become established or function on or near the root (Weller, 1988).

5.2. Environmental conditions which would affect expression of the bacterial activities

Another important negative factor is the inconsistent production or the *in situ* inactivation of the secondary metabolites that contribute to disease control. For effective biocontrol, production of these metabolites must coincide with the period of time when the plant is vulnerable to attack. In the rhizosphere, the temporal regulation of secondary metabolites is likely to be even more tightly controlled and to be dependent on the environment within the microsite (Duffy and Defago, 1999). For example, oomycin A biosynthesis was induced by glucose but inhibited by combinations of amino acids, all of which are found in root exudates (Gutterson, 1990). Further, both temperature and water potential affect expression of *afu*E, a key oomycin A

biosynthetic gene, in the rhizosphere (Gutterson *et al.,* 1986). Since environmental factors in the rhizosphere may influence the expression of these gene and thus influence the biocontrol activities of the strains, we will have to increase our knowledge on the growth conditions in the rhizosphere.

Ownley *et al.* (1992) found that the performance of *P. fluorescens* 2-79 against take-all varied considerably in ten soils. This indicates that the effectiveness of a given biocontrol agent may be restricted to specific locations due to the effects of soil and climate. Some soil variables like ammonium-nitrogen, sulfate-sulfur, zinc, soil pH, extractable and soluble sodium, and percent sand were positively correlated for biocontrol activity; other variables such as cation exchange capacity, percent silt, percent clay, percent organic matter, manganese, and iron were positivley correlated with disease severity (Thomashow and Pierson, 1991). Therefore, the appropriate temporal and spatial regulation of metabolite(s) synthesis by the biocontrol agent is essential to improve the effectiveness of biological control. Laboratory studies are increasing our knowledge of the environmental factors that may influence the production of metabolite(s) at the appropriate microsites in the rhizosphere; however, a large gap remains in our understanding of the interactions between rhizosphere exudates and microbial metabolite production.

Many organims suppress disease effectively in the laboratory but fail to do so in the field. The interaction of the biocontrol agent with the microbial community may provide clues to explain the failures. The limitations of the biocontrol agents can be addressed by enhancing biocontrol through manipulation of the environment, physiological and genetic enhancement of the biocontrol mechanisms, manipulation of formulations, using mixtures of beneficial organisms, and integration of biocontrol with other alternative methods that alone do not provide adequate protection but in combination with biocontrol provide additive or synergistic effects.

6. Epilogue

The prospects for control of plant diseases by using the use of biological control agents are promising and proven to be excellent methods.

However, the successful biocontrol of plant disease requires an understanding the intricate array of interactions at molecular and ecological levels that will make possible the rational development of biocontrol agents. Application of genetic analysis to microorganisms involved in biocontrol has led to substantial progress in understanding the microbial metabolites and regulatory genes involved in biocontrol. Molecular methods developed for the study of microorganisms in their environments are key tools for the study of the influences of the microbial community on biocontrol. The integrated use of genetic, molecular, and ecological approaches will form the basis for significant future advances in biocontrol research. The additional effort in these three areas would be essential for developing a more complete understanding of biocontrol and for making practical use of biocontrol strategies for plant diseases. The subject is so challenging that one should approach it hopefully and with optimism, not with skepticism and doubt. The advantages would include better yield, lower costs and reduced dependence on fungicides and fertilizers. It would be expected that natural means of pathogen control would be favored over the use of synthetic fungicides.

7. References

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13

Prospects of Arbuscular Mycorrhiza in Sustainable Management of Root- and Soil-Borne Diseases of Vegetable Crops

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ABSTRACT: Vegetable crops are highly prone to a number of root and soil borne diseases causing great losses in yield and quality. Indiscriminate use of fungicides and pesticides in controlling the diseases has polluted the environment and produce. Thus, there is need of proper management of these diseases at reduced doses of pesticides to sustain the vegetable production. Biological control of plant diseases is one of the viable alternatives in sustainable agriculture. Arbuscular mycorrhizal fungi are associated with most agricultural crops and provide protection against soil-borne diseases. The degree of reduction in diseases development by AM fungi varies with the combination of host-AM-environmental conditions. Two groups of major soil borne pathogens of vegetables have been described here, namely nematode and fungi. Generally inoculation with AM fungi had a negative effect on growth of pathogens, but most of the reports for nematode diseases are on migratory and sedentary endoparasitic nematodes. The prophylactic action of AM may further be improved with the integration of antagonistic rhizosphere microbes to improve plant health. The mycorrhizal efficacy also varies by alteration in soil nutrition, mycorrhizosphere, inoculation sequences and modification of cultural practices. Number of mechanisms are involved in controlling the pathogen by mycorrhizal roots such as exclusion of pathogen, lignifications of cell wall, changed P nutrition, exudation of low molecular weight compounds *etc*.

1. Introduction

Vegetables are an important component of the human diet providing essential vitamins and fibres. Vegetable crops being rich in moisture and nutrients are more susceptible to disease as compared to other commodities. Apart from abiotic stress a number of biotic factors like fungi, bacteria, virus, and nematode are responsible for causing significant losses in terms of yield and market value caused by

dystrophy and malformation. The disease scenario is dynamic with arrival of new promising varieties. Introduction of exotic hybrids, frequent unchecked movement of genetic materials and their intensive cultivation have further aggravated the situation. In this context, integrated disease management (IDM) in vegetable crops for long-term sustainability of poor vegetable growers as well as vegetable crops of the country is inevitable. Its effectiveness in vegetable crops is par excellent beyond doubt. However, it has man made limitations, which can be easily solved by massive and collective efforts of researchers, teachers, extension workers, farmers, industrialists, administrators, and legislatures. For appreciable gains the proposed IDM approach must be applied on a communicative basis as wide area approach on community basis. Particularly in integrated disease management, the most important factor is to develop and adopt the package for multi disease approach for the whole year. A complete cyclic calendar must be developed and recommended on the basis of major diseases occurring in that specific agro-climatic condition.

The chemical pesticides in addition to causing non-target effects are becoming expensive year after year and loosing their effectiveness as a result of co-evolution and development of resistance to pathogen. Most of the new generation pesticides are systemic in their mode of action leading to certain level of toxicity in the plant system and thus is hazardous to health. Further, it disturbs the ecology of the microbial diversity in ecosystem (Mukerji, 1999). All these factors lead to new dimension for research in integrated disease management (IDM) which can make an important contribution to sustainability and safe vegetable production (Kumar and Mukerji, 1996). In recent years the research, demonstration, commercialization and practical use has been taken in real application in agriculture.

Increasing knowledge and concern about the environmental consequences of pesticide applications have aroused interest in alternative methods of plant protection. The roots of most plant species live in symbiosis with certain soil fungi by establishing mycorrhizae. The fungus biotrophically colonizes the root cortex and develops an extramatrical mycelium that helps plant to acquire mineral nutrients from soil (Harley and Smith 1983). Plant producers are showing increasing interest in the role of mycorrhizal fungi as bio-protectors, bio-stimulators or biofertilizers (Azcón-Aguilar and Barea, 1997). It is known that plants benefit from fungal presence in roots when

exposed to a broad range of abiotic stresses like high salinity, drought, heavy metal accumulation in soil (Gernns *et al,* 2001).

Mycorrhiza inoculation has additional advantage as they are effective even against soil-borne pathogens, which are difficult and expensive to manage through chemical and physical treatments (Azcón-Aguilar and Barea, 1996, 1997; Cordier *et. al.,* 1997; Singh, *et. al.,* 2000). Mycorrhizal plants are often less colonized by pathogens and disease incidence (Torres-Barragan *et. al.,* 1996). Hence, mycorrhization has been proposed as an alternative for the management of soil-borne pathogens (Dehne, 1982; Perrin, 1990). Generally, AM fungi cause few changes in root morphology, but the physiology of the host plant changes significantly (Sharma and Adholeya, 2000). The improved potential for mineral uptake from the soil accounts for changes in the nutritional status of the host tissues, which, in turn, changes the structural and biochemical aspects of root cells. This can alter membrane permeability and thus the quality and quantity of root exudation. Altered exudation induces changes in the composition of microorganisms in the rhizospheric soil, which could be known as 'mycorrhizosphere' (Bansal and Mukerji, 1994). The net effect of these alterations is a tolerant plant better able to withstand environmental stress and plant diseases (Linderman, 1988). To evaluate the influence of AM fungi on diseases incidence and development, the variable factors, namely plant pathogens, the symbiotic fungus, and environmental conditions have been considered. Mostly, the interactions between pathogen and the symbiont are mediated by the host. The characterization of these interactions should therefore include information on the mechanisms involved, because interactions vary with specific host-symbiont-pathogen combination; it is difficult to make generalizations on the effect of AM fungi on diseases.

The purpose of this review is therefore to attempt (i) to analyze the potentiality of AM fungi and mechanisms involved in the biocontrol of root and soil borne pathogens of vegetable crops and, (ii) to exploit the possibilities of mass production of AM fungi.

2. Economic losses

Majority of the vegetable diseases are caused by fungi, affecting nursery seedling stage to harvest and spoilage during transit, storage and

marketing. Globally estimates indicates 65 million tones pre harvest losses out of which, disease alone accounts for 10.5 %. A maximum loss of 44 million tones is reported only from developing countries. Post harvest losses are equally important because most of the vegetables are highly perishable in nature and easily colonized by microorganisms. Post harvest losses are of much concern and estimated to about 20-40 % of the total fruit and vegetable production costing more than Rs. 30000 million annually.

3.   Changing pest scenario in vegetables and status of IPM in vegetable crops

Introduction of hybrids in vegetable crops has created several new disease problems like *Pseudocercospora* leaf blight in tomato and brinjal hitherto unknown in India. Intensity of many diseases such as enation leaf curl in okra (OLCV) and chilli leaf curl, gummy stem blight, *Didymella* leaf blight and viral diseases of cucurbits increased tremendously. Alternaria leaf blight in cole crops increased throughout the country due to hybrids. Intensive and monoculturing of vegetables also increased the disease pressure of soil-borne pathogens. *Sclerotium*, *Sclerotinia* and *Rhizoctonia* have become ubiquitous affecting almost all vegetable crops and becoming serious every year. The off-season vegetable growing practices have prolonged the survival period of pathogens in the field. Collar rot severity and its inoculum in soil increased due to susceptible F_1 materials of tomato. Seed-borne diseases like black rot of cole crops and their inocula in vegetables increased throughout the country (Pandey and Pandey 2002).

It is very important to know the survival stages of pest, their pathways of infection, congenial condition for disease development and detailed epidemiology in the selection of different components of integrated pest management (IPM). The cultivation practices in vegetable crops are entirely different from other food crops. It differs in terms of short duration, dense crop canopy, high nutrient requirement, more water requirement, delicate nature of the crop, intensive weed management, indeterminate fruiting behavior, frequent periodical harvesting of fruits, maximum number of disease and insect problems. The indiscriminate and intensive use of pesticides increases

the risk of resistance in pests and diseases (Table 1). Many vegetable growers are themselves responsible for reducing effectiveness of pesticide by increasing the application frequency, using higher doses above the recommended dose and selecting extremely toxic, systemic, single site acting pesticide. I PM is based on ecosystem approach and must give importance for pest population, natural enemies, environmental factors, biosphere, yield economics and social acceptance. From 1950s onwards IPM got a lot of attention and it is considered as the only rational approach to provide long-term sustainable solution to pest problem. However, the adoption of IPM technology by farmers is slow and the main constraint throughout the world is transfer of IPM technology at grass root level (Cameron *et. al.,* 1982, Upadhyay *et. al.,* 1998). Soil solarization by mulching polythene sheets prior to planting of eggplant and tomato reduced *Verticillium* wilt by 25-95%, controlled weeds, improved plant growth stands and increased yield (Katan *et. al.,* 1976). Plastic mulching during soil solarization effectively controlled southern blight of tomato caused by *Sclerotium rolfsii* and the sclerotia were unable to survive on soil surface up to the depth of 5 cm (Tu *et. al.,* 1991). The present status of IPM in vegetable is much advanced and in practice than any other crops and gradually gaining momentum. The vegetable growers and consumers realized the ill effects of pesticides. Presently biopesticides like *Pseudomonas*, *Bacillus, Aspergillus, Verticillium* and *Trichoderma* are being used for management of diseases in many vegetable crops (Table 2). Several botanicals like neem formulations, pongamia oil and karanj are applied in soil as well as for foliar spray to manage disease spreading vectors. The demand of biopesticides by vegetable growers has enhanced national and international market. Soil solarization and biocontrol is now an integral component for nursery disease management and soil borne diseases of vegetable crops (Table 3). Green manuring followed by *Trichoderma* application in soil is the best method for the management of soil-borne diseases. Similarly, summer fallowing and ploughing accompanied by one irrigation encourages germination of resting spores, sclerotia, weeds and activate insects, nematodes larvae which gives very good IPM strength in vegetables. Foliar spray of *Trichoderma* sps. in potato against late blight and thorough spray on all ground portions in pointed gourd gave very good control of *Phytophthora* blight. Similarly one

TABLE 1

Diseases and pests of increased resistance to pesticides

Pathogen	Pesticide
Botrytis cinerea	Benzimidazole (benomyl)
	Dithioconbamates
	Thiophamate methyl
Cladosporium spp.	Benzimidazole
	Dithioconbamates
Leveillula taurica	Pyrimidin (Fenarimol)
Phytophthora infestans	Metalaxyl
Rhizoctonia solani	Benzimidazole
	Dithioconbamates
Sclerotinia spp.	Benzimidazole
	Dithiocarbamates
Verticillium dahliae	Benzimidazole
	Thiophanate methyl

TABLE 2

Biocontrol agents available in India.

Trade product	Bioagent	Manufacturer
Antagon Combi, Antagon TV	*Trichoderma viride*	Green Teen Agro Products, Rajaji Road, Coimbatore
Trichogaurd	*Trichoderma viride*	Anu Biotech International Tigocon Road, Faridabad 121 002
Niprot	*Trichoderma viride*	Pest Control India Ltd., Biocontrol Research Laboratory, Post Box No. 3228, Bangalore - 560 032
Biomonas	*Pseudomonas fluorescence*	Biotech International, New Delhi
Tridhodex XP	*Trichoderma viride, Paceilomyces* sp.	Excel Industry Ltd. Bombay
Bioderma	*Trichoderma viride*	Biotech International, New Delhi

TABLE 3

Examples of yield increase by solar heating (Katan, *et al.* 1976)

Crop	Pathogen	Increase over control (%)
Potato	*Verticillium dahliae* and *Pratylenchus thornei*	35
Onion	*Pyronchaeta terrestris*	60-125
Tomato	*P. lycopersici*	100-300
Tomato	*P. lycopersici*	245
Tomato	*P. lycopersici*	140-350
Eggplant	*V. dahliae*	215
Carrot	*Orobanche aegyptiaca*	-

of the *Aspergillus niger*-V strain along with sticker and molasses was sprayed on tomato against *Alternaria* blight and gave satisfactory control. At present, success of IPM will depend on vegetable growers when they will gradually shift their cropping pattern from intensive, pesticide based, modern cultivation to biopesticides based, extensive, broad spectrum, sustainable vegetable production.

4. Biological control in vegetable diseases

This broad-spectrum definition includes practices that create an environment favorable to antagonists, mass introduction of antagonists, non-pathogenic strains or other beneficial organisms (Kumar *et. al.,* 1997; Mukerji and Garg, 1988a,b). Three antagonistic mechanisms are involved in biological control of soil-borne plant pathogens *viz.,* competition, antibiosis and mycoparasitism. Competition occurs when there is a demand of two or more microorganisms for the same resource in excess of the immediate supply. This is most potent mechanism in the control of *Fusarium oxysporum* f. sp. *melonis* by *Trichoderma harzianum.* Antibiosis occurs when the production of toxic metabolites or antibiotics (volatile or non-volatile) produced by the biocontrol agent has a direct effect on another organism. The antagonists like *T. virens* produces gliotoxin, viridin and trichodermin produced by *T. viride*, *T. polysporum* is effective against *Pythium ultimum* and

Macrophomina phaseolina. Mycoparasitism or hyperparasitism describe the phenomenon of one fungus parasitising another by the process of chemotropic growth, recognition, attachment and degradation. Production of lytic enzymes, chitinases and β-1, 3 glucanoses are the most important for lysis of pathogen cell wall. Soil pathogen causing root rot, damping off, collar rot and wilt in different vegetable crops are the major target of biocontrol agents but many foliar pathogen like *Alternaria solani* and *Colletotrichum capsici* were also suppressed by the *Aspergillus niger* V-isolate (Pandey and Pandey, 2002). Apart from pathogenic fungus, the antagonistic fungus also kills some parasitic nematodes infecting vegetables. The important target pathogen are *Pythium* spp., *Sclerotium rolfsii*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium* spp., *Phytophthora* spp., *Phoma* spp., *Sclerotinia sclerotiorum*, *Aspergillus* spp, *Botrytis* spp., *Armillaria* spp., *Verticillium* spp., *Erysiphe* spp. (Table 4).

TABLE 4.
Prevalence of fungal diseases of vegetable crops

Crop	Disease	Pathogen	Severity (%)	Average incidence (%)
Brinjal	White rot	*Sclerotium rolfsii*	5-22	8.7
	Collar rot	*Sclerotinia sclerotiorum*	10-30	20
	Root rot	*Rhizoctonia solani*	5-20	4.2
	Rhizopus fruit rot	*Rhizopus stolonifer*	2-20	5.2
	Wilt	*Fusarium solani*	1-2	1
	Alternaria blight	*Alternaria solani*	15-90	40
Tomato	White rot	*Sclerotium rolfsii*	5-10	5
	Collar rot	*Sclerotinia sclerotiorum*	10-40	20
	Fruit rot	*Rhizoctonia spp.,* *Cladosporium spp.,* *Pythium spp.*	5-20	15
	Black leaf mold	*Pseudocercospora fuligena*	0-3	<1
	Wilt	*F. oxysporum* f. sp. *lycopersici*	0-2	<1
Chilli/ Capsicum	Phytophthora blight	*Phytophthora capsici*	5-10	8
	Collar rot	*Sclerotinia sclerotiorum*	2-5	3
	White rot	*Sclerotium rolfsii*	0-2	1
	Root rot	*Rhizoctonia solani*	2-8	5

Table 4 cont.

Cowpea/Pea/ Beans (Legumes)	Leaf blight	*Pseudocercospora cruenta, Cercospora cruenta*	20-90	60
	Ascochyta blight	*Ascochyta phaseolorun*	5-15	8
	Root rot/Ashy stem rot	*Rhizoctonia bataticola*	2-15	5
Cole crops	White rot	*Sclerotinia rolfsii*	2-10	5
	Root rot/wire stem	*Rhizoctonia solani*	5-15	8
	Black leg	*Phoma lingam*	2-5	2
Root crops	Collar rot	*Sclerotium rolfsii*	2-10	4
	White rot	*Sclerotinia sclerotiorum*	2-3	2
	Charcoal rot	*Macrophomina phaseolina*	5-15	8
	Downy mildew	*Peronospora destructor*	10-25	10
Elephant foot, yam (Suran)	Collar rot	*Sclerotinia sclerotiorum*		

4.1. Potential biocontrol agents

Several antagonists have been isolated and many of them are employed in the management of soil-borne pathogen. Some of the antagonists are highly specific to a particular pathogen but most of the fungal antagonists have broad-spectrum host range on which they parasitise. Following biocontrol agents are generally used for disease management (Cook and Backer, 1989).

Fungi   -   *Trichoderma* spp., *Aspergillus niger*, *A. flavus*, *Pythium nannum*, *Trichothecium* spp., *Paecilomyces lilacinus*, *Penicillium* spp., *Myrothecium* spp., *Corticium* spp., *Pythium oligandrum*, *Peniophora gigantea*, *Candida oleophila*, *Sporidesmium sclerotivorum*, *Coniothyrium minitans*, *Ampelomyces quisqualis*, *Chaetomium* spp., *Cladosporium* spp., *Fusarium semitectum*, *Tuberculina* spp., *Phialophora* spp., *Catenaria* spp., *Verticillium* spp.

Bacteria   -   *Pseudomonas* spp., *Agrobacterium radiobactor*, *Bacillus* spp.

Actinomyces -   *Streptomyces griseus, S. rimosum*

Trichoderma sps. have been extensively exploited in biocontrol of soil-borne disease of vegetables, because of their soil inhabitant nature and also because they are antagonistic *e.g. T. harzianum*, *T. viride*, *T. virens*, *T. koningii*, *T. hamatum*, *T. polysporum*, *T. aureoviride*, *T. piluliferum*, *T. pseudokoningii* and *T. longibrachiatum* (Cook and Backer, 1989). *Trichoderma* sps. can be easily isolated from any soil but it is necessary that it should be resident of a particular soil-ecosystem for effective use in that area as biocontrol agent.

5.   Management of root-and soil-borne diseases using arbuscular mycorrhizal (AM) technology

5.1   Potential of AM fungi in control of root- and soil–borne fungal pathogens of vegetables

Several reviews have been written on AM and biological control of soil-borne fungal diseases of vegetables (Azcón-Aguilar, 1996; Bagyaraj, 1984; Dehne, 1982; Hooker, *et. al.,* 1994; Jalali and Jalali, 1991; Linderman, 1994; Mukerji *et. al.,* 1997; Sharma and Adholeya, 2000; Sharma and Johri, 2002; Sharma *et. al.,* 1992 and Xavier and Boyetchko, 2002). Main conclusions that can be drawn are: (i) AM associations can reduce damage caused by soil-borne plant pathogens, (ii) the abilities of the AM symbioses to enhance resistance or tolerance in roots are not equal for the different AM fungi so far evaluated, (iii) protection is not effective for all pathogens, and (iv) protection is modulated by soil and other environmental conditions. Hence, it can be speculated that interactions between different AM fungi and plant pathogens will vary with the host plant and the cultural conditions.

Since arbuscular mycorrhizal fungi are established in the roots of plants, research on mycorrhizae-disease incidence interactions has been concentrated on disease caused mainly by soil-borne pathogens. Although most of the experiments differ in approach and methods, a summary of the results is given in Table 5.

Most of the studies have concentrated on the effect of AM fungi on improved nutrition and growth of plants (Stribley, 1990; Yao *et. al.,* 2002). A number of hypotheses have been proposed, including

TABLE 5

Influence of arbuscular mycorrhizal fungi (AMF) on control of
vegetable fungal pathogens

Fungal pathogen	Host plant	Disease incidence	Reference
Rhizoctonia solani	Potato	-	Yao *et. al.,* 2002
		-	Bodker *et. al.,* 2002;
Aphanomyces euteiches	Pea	-	Rosendahl 1985
Sclerotium cepivorum	Onion	-	Torres-Barragan 1996
F. oxysporum f. sp. *cepa*		-	Dehne 1982
F. oxysporum f. sp. *cumini*	Cumin	+	Champawat 1991
F. oxysporum f. sp. *lycopersici*	Tomato	-	Dehne and Schönbeck 1979
F. oxysporum f. sp. *lycopersici*		-	McGraw and Schenck 1981
F. oxysporum f. sp. *radicis lycopersici*		-	Caron *et. al.,* 1986b
Phytophthora nicotianae		-	Datnoff *et. al.,* 1995
f. sp. *parasitica*		-	Trotta *et. al.,* 1996
Phytophthora parasitica		-	Pozo *et. al.,* 2002
F. oxysporum f. sp *asparagi*	Asparagus	-	Wacker *et. al.,* 1990 Matsubara *et. al.,* 2001
Colletotrichum lindemuthianum	French bean	+	Schönbeck and Dehne 1979
Phoma terrestris	Onion	......	Becker 1976
Pyrenochaeta terrestris		-	Safir 1968
Pyrenochaeta lycopersici	Tomato	-	Bochow and Abou-Shaar 1990
Pythium aphanidermatum		-	Hegde and Rai 1984
Sclerotium rolfsii	Chilli	-	Sreenivasa *et. al.,* 1992
Sclerotium cepivorum	Garlic	.....	Prados-Ligero *et. al.,* 2002
Rhizoctonia solani	Tomato	-	Casiolata and Melo 1991

enhanced nutritional status of the host plant (Sharma *et. al.,* 1992), production of antimicrobial compounds (Morandi and Gianinazzi-Pearson, 1986) and changes in the microbial community in the rhizosphere around mycorrhizal roots (Meyer and Linderman, 1986a).

Increased phosphorus (P) assimilation has been proposed to explain higher tolerance of mycorrhizal plants to pathogens (Azcón-Aguilar and Barea, 1996; Zambolim and Schenck, 1983). However, there are a number of contradictory reports (Hooker *et al* 1994; Linderman, 1994). Higher disease resistance by mycorrhizal (*Glomus mosseae*) tomato plants towards soil-borne root pathogen *Phytophthora nicotianae* var. *parasitica* has also been shown (Trotta *et al* 1996). They suggested that effect of P nutrition were only apparent in the form of stimulated plant growth. Overall, P appears to have lesser effects on *Phytophthora* diseases than other chemical factors (Schmitthenner and Canaday, 1983). However, other authors observed no effect of P fertilization on pathogen population (Caron *et. al.,* 1986b). On the other hand, Wacker *et. al.* (1990) showed that *Asparagus officinalis* plants inoculated with *Glomus fasciculatum* under high phosphorus concentration in soil had significantly lower rating of the disease caused by *F. oxysporum* than control plants grown in low level of phosphorus. The rhizosphere population of pathogen was found to be lowest in high P soil regardless of AM status. It is apparent from many reports that AM fungi can lead to lower disease incidence on mycorrhizal plants (Caron, 1989; Perrin, 1990; St-Arnaud *et. al.,* 1994). Mycorrhizal mediated tomato plants by *Glomus intraradices* significantly reduced the population of *Fusarium* root-rot caused by *Fusarium oxysporum* f. sp. *radices-lycopersici* (Caron *et. al.,* 1986b). St-Arnaud *et al* (1994) observed that inoculations of substrate with *G. intraradices* reduced the populations of *Pythium ultimum* on *Tagetes patula*. They showed that the extent of colonization bearing arbuscules or vesicles of AM fungi was unrelated to P nutrition and to the observed reduction of *P. ultimum* in the roots or in the substrate. Very recently, Bodker *et. al.,* (2002) showed that mycorrhization of field grown pea significantly affects the life cycle of *Aphanomyces euteiches* (root rot of pea). The rate of increase in pathogen infection potential showed negative correlation with the levels of indigenous AM population in the soil. Torres-Barragan *et. al.* (1996) also showed that onion plants inoculated with AM fungi reduced the white rot incidence and delayed the disease development by two weeks over control plants. In addition, the onion roots having higher colonization and spore density in the rhizosphere showed lower

disease and *vice versa*. There are reports that contradict that high level of mycorrhization is necessary to induce protection against pathogens (Graham and Menge, 1982; Smith, 1988; Smith *et. al.,* 1986) and agrees with the results of Caron *et al* (1986a), who could protect tomato plants against *F. oxysporum* even at very low colonization levels. Dugassa *et. al.* (1997) reported that the influence of AMF symbiosis on plant health depends more on host-plant and pathogen genotype than on AM colonization level. Mycorrhizal inoculation with either *Glomus intraradices* or *G. etunicatum* on plantlets of potato cultivar Goldrush enhanced growth and yield and improved resistance/tolerance to *Rhizoctonia solani* infection. In natural conditions as well as through inoculation, most species or isolates of AM fungi can establish symbiosis with very wide host range including potato (Bhattarai and Mishra, 1984). However, many studies have demonstrated that the AM fungi-plant cultivar combination influences plant responses (Yao, 1996). The influence exerted by AM fungi by stimulating plant defense reactions was investigated using an in *vitro* system in which RiT-DNA-transformed carrot roots were infected with *Fusarium oxysporum* f.sp. *chrysanthemi*. In the non-inoculated roots, the pathogen multiplied abundantly through much of the tissue, including the vascular stele, whereas in mycorrhizal plants its growth was restricted to the epidermis and the outer cortex (Benhamou *et. al.,* 1994).

The reduction in the infection by *F. oxysporum* in mycorrhizal roots (inoculated with *G. mosseae*) of tomato and *Capsicum* was reported (Al-Momany and Al-Raddad, 1988). Tomato plants inoculated with *G. mosseae,* showed only 11% incidence of Fusarium wilt as against 45% in non-mycorrhizal plants (Ramraj *et.* al., 1988). Rosendahl and Rosendahl (1990) showed that inoculation of cucumber seedling with *G. etunicatum* and *Glomus* sp. before or simultaneously with *Pythium ultimum* increased the survival of the seedlings and saved the plants from damping off.

5.2. Arbuscular mycorrhizae in the suppression of soil-borne nematodes

The association of AM fungi with plant nematodes and the beneficial effect of mycorrhizal symbiosis on plant growth has led to

investigations into the potential of AM fungi to limit yield losses due to nematodes (Table 6). Mycorrhiza–nematode interaction can be quantified by measuring the effect on fungal colonization of roots or sporulation and nematode attraction to roots, penetration, or subsequent development and reproduction. Plant responses to concomitant infection can be assessed on plant growth or yield, by either stimulation from mycorrhizal development or suppression from nematode infection. The possible effects of interactions between plant-parasitic nematodes and AM fungi on each component are summarized in Table 7, where no changes are evident, the interaction is considered to be neutral. An interaction is rated positive if AM fungi offset nematode damage to plants; since the response of a plant to the endophyte is rarely enhanced in the presence of nematodes. AM fungi

TABLE 6
Influence of arbuscular mycorrhizal fungi (AMF) on nematode diseases of vegetable crops (Sharma and Adholeya, 2000)

Nematode	Host Plant	Incidence	Reference
Meloidogyne arenaria	Clover	-	Kassab and Taha (1990)
M. hapla	Carrot	-	Sikora and Schönbeck (1975)
M. hapla	Tomato	-	Cooper and Grandison (1986)
M. hapla		-	Cooper (1981)
M. hapla	Alfalfa	-	Grandison and Cooper (1986)
M. incognita	Cucumber	-	Priestel (1980)
M. incognita	Tomato	-	Bagyaraj *et. al.* (1979)
M. incognita	Tomato	-	Suresh *et. al.* (1995)
M. incognita		-	Sikora and Schönbeck (1975)
M. incognita		-	Suresh and Bagyaraj (1985)
M. incognita		-	Sharma *et. al.* (1994)
M. incognita	Eggplant	-	Rao *et. al.* (1998)
M. javanica	Tomato	-	Bagyaraj *et. al.* (1979)
M. javanica	Tomato	-	Al Raddad and Ahmad, 1995
M. incognita	Tomato	-	Talavera *et. al.* (2002)
Pratylenchus penetrans	Cucumber	-	Priestel (1980)
Rotylenchus reniformis	Tomato	-	Sitaramaiah and Sikora (1982)
Meloidogyne sp.	Egg plant	-	Hasan (2001)
	Lady finger	-	Hasan (2001)
	Tomato	-	Hasan, (2001)

- decrease ; + increase

TABLE 7

Possible effects of interactions between plant-parasitic nematodes and AM fungi (Hussey and Roncadori, 1982)

Type of interaction	Component	Effect on component
Neutral	Fungus	Root infection or sporulation not altered
	Host	Mycorrhizal stimulation of vegetative growth or yield not altered; nematode suppression of vegetable growth or yield not offset
	Nematode	Attraction to roots, penetration, or subsequent development and reproduction not altered
Positive	Fungus	Root infection or sporulation increased
	Host	Nematode suppression of vegetable growth and yield offset
	Nematode	Attraction to roots, penetration or subsequent reproduction and development suppressed
Negative	Fungus	Root infection or sporulation suppressed
	Host	Yield response to mycorrhiza suppressed
	Nematode	Attraction to roots, penetration or subsequent reproduction and development increased

however, could have an antagonistic effect on nematodes, and such an effect could have either a physiological or physical basis (Hussey and Roncadori, 1982).

Symptoms of nematode infection are generally reduced and often, but not always, nematode populations are also reduced (as indicated by number of galls, juveniles or eggs per unit root length) (Hussey and Roncadori, 1978). Inoculations of tomato transplants with *Glomus fasciculatum* significantly reduced root penetration by juveniles and the development of *Rotylenchulus reniformis* compared to controls. Development of the gelatinous matrix was delayed and fewer eggs per eggmass were produced on inoculated plants. The number of galls formed by *Meloidogyne incognita* on tomato was significantly lower in AM-inoculated tomato plants. However, the

AM colonization did not prevent the penetration by the larvae (Suresh *et. al.,* 1985). Baghel *et. al.* (1990) showed that inoculation of *G. mosseae* stimulated the growth of *Citrus jambhiri* seedlings. Simultaneous inoculations of AM fungus and the citrus nematode, *Tylenchulus semipenetrans* partly neutralized the adverse effect of the nematode. Pre-inoculations of *Piper nigrum* cv. *panniyar* roots with *G. fasciculatum* or *G. etunicatum* significantly reduced the root-knot index (*Meloidogyne incognita*) by 32.4 and 36.0% respectively and also the growth of piper plants improved (Shivaprasad, Personal Communication).

Krishna Prasad (1991) reported that the percentage of root-knot nematode (*M. incognita*) infestations in tobacco (*Nicotiana tabaccum*) seedlings was 67.5% at 50 days and 95% at 75 days after sowing in non-mycorrhizal plants and 48% to 52% at 50 days and 73% at 75 days after sowing in mycorrhiza (*G. fasciculatum*) inoculated plots. The number of galls, egg masses per plant, and eggs per egg mass of infested plants was reduced by 61-89% as a result of AM inoculation. Transplanting of mycorrhizal tobacco seedlings into root-knot nematode infested soil showed improved growth and yield compared to non-mycorrhizal plants.

Al-Raddad and Ahmad (1995) showed that pre-inoculations of *G. mosseae* significantly reduced root knot infections and reproduction of root-knot nematode in tomato roots. The ability of mycorrhizal plants to grow well despite infection by nematodes is generally considered to be the principal affect of mycorrhizal fungi or the interaction of host plants with parasitic nematodes (Hussey and Roncadori, 1982). Furthermore, a comparison of mycorrhizal and P-fertilized non-mycorrhizal plants showed that the latter are more susceptible to nematode attack indicating the likely involvement of factors other than P nutrition in the interactions (Smith, 1988). On the other hand, mycorrhizal inoculations of tamarillo (*Cyphomandra betacea*) against root-knot nematode, *M. incognita* could not be duplicated by adding P fertilizer and was not therefore due to merely improved P nutrition of the host (Cooper and Grandison, 1987). Heald *et. al.,* (1989) showed that *M. incognita* suppressed the growth of non-mycorrhizal *Cucumis melo* plants by 84% as compared to 21% in AM inoculated plants at 50 mg/g P. A similar trend was observed

in soil with 100 mg/g phosphorus. Pre-inoculations of *G. fasciculatum* in tomato var. Pusa ruby significantly reduced the number of galls/plant, egg masses per plant and eggs per egg mass due to *M. incognita*. They also observed that the offset of AM fungi on reducing root-knot nematode was not the same in pots and in the field. Nematode population of AM-mediated tomato grown plots was reduced up to 36% where as in the pots the nematode reduction was 58% (Sharma *et. al.,* 1994).

The sequence in which plants become mycorrhized and infected by nematodes may affect the interaction between these organisms on certain plants. Pre-inoculation of tomato and other transplanted crops with AM fungi to allow this slow growing symbiont to become established in the roots before the attack of *M. incognita* resulted in fewer juveniles penetrating, and developing to maturity in roots of mycorrhized plants than in roots of control plants (Sharma *et. al.,* 1994). Studies conducted by several workers demonstrate that pre-inoculations of AM fungus suppressed nematode reproduction and development in roots to a greater degree when compared to plants inoculated simultaneously with both the organisms (Jain and Sethi, 1987; Suresh and Bagyaraj, 1984; Taha and Abdel-Kader, 1990). Apart from reduced penetration of roots by nematodes, mycorrhization also results in reduced development of the pathogen (Sharma and Johri, 2002). Priestel (1980) reported specific decrease in reproduction rate of larvae of *Meloidogyne*, which resulted in strong reduction in egg production. This observation assumes great significance in field and helps to explain their changed population dynamics in the mycorrhizal root system (Sitaramaiah and Sikora, 1982).

Azcón-Aguilar and Barea (1996) concluded that AM-induced increase in resistance or a decrease in susceptibility required the pre-establishment of AM and extensive development of the symbiosis before the pathogen attack.

5.3. Mechanisms of AM in controlling root pathogens

Mechanisms that could play role in protective activity by AM fungi include improvement of plant health, root damage compensation,

competition for space, changes in root morphology, changes in mycorrhizosphere microbial community and activation of plant defense mechanisms. The role played by AM fungi in biological control of plant diseases has been the subject of several reviews (Azcón-Aguilar and Barea, 1996; Bagyaraj, 1984; Caron, 1989; Jalali and Jalali, 1991; Mukerji, 1999; Sharma and Johri, 2002) but mixed responses and interpretations have precluded any clear conclusion that AM fungi always suppress plant diseases. Such inconsistencies should be expected, however, considering the diverse experimental approaches and the use of different AM fungi on different hosts in different soils (Schenck, 1987). Mycorrhizal fungi are biocontrol agents against plant diseases primarily by means of stress reduction (Baker, 1986; Cook and Baker, 1982).

5.3.1. Improved nutritional status of host plant

AM fungi are known to improve plant growth and nutrient absorption and physiological responses of the host to environmental stresses resulting in more resistance or tolerance to pathogen attack (Azcón-Aguilar and Barea, 1996). Many of these, especially nutrient factors are related to P physiology and nutrition (Graham *et. al.,* 1981, Hayman, 1982, Pacovsky *et. al.,* 1986). AM fungi enhance root growth, expand absorptive capacity and affect cellular processes in roots (Hussey and Roncadori, 1982; Smith and Gianinazzi-Pearson, 1988). These mycorrhiza-induced compensatory processes may explain the increased tolerance of mycorrhizal and P- fertilized plants because they can compensate for loss of root mass or function caused by pathogens (Linderman, 1994) including nematodes (Pinochet *et. al.,* 1996) and fungi (Cordier *et. al.,* 1996).

In addition to P, AM fungi can enhance the uptake of Ca, Cu, Mn, S and Zn (Smith and Gianinazzi-Pearson, 1988, Pacovsky *et. al.,* 1986). For example, nematode-damaged plants frequently show deficiencies of B, N, Fe, Mg and Zn (Good, 1968). Thus, AM fungi may increase tolerance to pathogens by increasing the uptake of essential nutrients other than P that would be deficient in a non-mycorrhizal plant. Caron *et. al,* (1986a,b,c) compared responses between AM and non-AM tomato plants with a relatively low P threshold requirement to root and crown rot disease caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Added P did not reduce disease incidence severity and pathogen populations in rhizosphere of non-AM plants but did so with AM plants, even though plant growth and tissue P were not different in the two treatments. This indicates indirect contribution to control pathogens through altered root morphology and nutrition, both by mycorrhiza growing out into the soil and increasing the absorbing surface of roots and by the maintenance of root cell activity through arbuscule formation (Cordier *et. al.,* 1996).

5.3.2. Competition for host photosynthates and infection site

It has been proposed that both AM fungi and pathogen growth depends largely on host photosynthates and carbon compounds reaching the roots (Linderman, 1994; Smith, 1987). When AM fungi have primarily access to photosynthates, the higher carbon demand may inhibit pathogen growth. However, there is little or no evidence that competition for carbon compounds is a generalized mechanism for pathogen suppression activity of AM symbiosis. Mechanisms affecting nematode activities in a mycorrhizal root systems that are not related to improved P nutrition or increased photosynthesis may involve utilization of photosynthates by the AM fungus at the expense of nematode reproduction or the conversion of carbohydrates - received from the host into forms not usable by the nematode. This contrasts with mass flow and diffusion of P through soil and root symplast on a non-mycorrhizal root (Bieliski, 1973). Dehne (1982) indicated that fungal root pathogens could occupy root cortical cells adjacent to those colonized by AM fungi, indicating a lack of competitions. It has been suggested that nematode pathogens, on the other hand, require host nutrients for reproduction and development and direct competition with AM fungi has been hypothesized as a mechanism of their inhibitors (Dehne, 1982, Smith, 1988). Since AM fungi, soil-borne fungal pathogens, and plant parasitic nematodes occupy similar root tissues, direct competition for space has been postulated as a mechanism of pathogen inhibition by AM fungi (Davis and Menge, 1980; Hussey and Roncadori, 1982; Linderman, 1985).

5.3.3. Morphological and anatomical changes in root system

AM fungi have been shown to induce remarkable changes in root morphology as well as in the anatomy (Atkinson *et. al.,* 1994). Lignification is reported to prevent penetration of mycorrhizal plants by pathogens. Dehne *et. al.* (1978) showed increased lignification of cells in the endodermis of mycorrhizal tomato and cucumber plants, and speculated that such responses accounted for reduced incidence of Fusarium wilt (*F. oxysporum* f. sp. *lycopersici*). A stronger vascular system in mycorrhizal plants increases flow of nutrients, imparts greater mechanical strength and reduces the effect of vascular pathogens (Schönbeck, 1979). Thus, most attention needs to be given to root system morphology in future because it could modify the infection dynamics of the pathogen as well as the pattern of resistance of AM roots to pathogen attack (Azcón-Aguilar and Barea, 1996).

5.3.4. Microbial changes in the mycorrhizosphere

AM formation induces changes in host physiology that can be decisive for root exudation patterns (Mandelbaum and Piche, 2000; Smith *et. al.,* 1994) and consequently, cause qualitative and/or quantitative alterations in microbial populations in the rhizosphere (Bansal *et. al.*, 2000; Meyer and Linderman, 1986b; Secilia and Bagyaraj, 1987). These microbial shifts were clearly time-dependent and dynamic. Meyer and Linderman (1986a) showed that the production of sporangium and zoospore by the root pathogen *Phytophthora cinnamomi* was

reduced in the presence of rhizosphere leachates from sweet corn and chrysanthemum. Similarly, populations of bacteria and actinomycetes in pot cultures of different AM fungi were quantitatively and qualitatively analyzed by Secilia and Bagyaraj (1987). They showed that pot cultures of *G. fasciculatum* harbored more actinomycetes antagonistic to *Fusarium solani* and *Pseudomonas solanacearum* than those of non-mycorrhized plants or plants colonized by other mycorrhizal species tested in the study. Other studies have indicated that pathogen suppression by AM fungi involved changes in mycorrhizosphere microbial populations. Caron *et al.* (1986a,b) showed a reduction in *Fusarium* population in the mycorrhizosphere soil of tomatoes and a corresponding reduction in root rot in AM plants when compared to non-AM plants, probably due to the increased antagonism in the mycorrhizosphere. They have also concluded that the reduction in disease was not due to P nutrition. Bartschi *et al.* (1981) reported that protection of host roots against *P. cinnamomi* root rot is due to pre-inoculation with mixed AM cultures taken from pot cultures. Further, Secilia and Bagyaraj (1987) demonstrated that the effect is due to a build up of antagonists in the pot cultures. Changes in mycorrhizosphere populations of species antagonistic to pathogens seem a likely explanation for many of the reported effects of AM on diseases. Therefore, the management of these interactions improving plant growth, and health in an integrated approach, should be one of the main objectives of sustainable agriculture (Barea and Jeffries, 1995; Bethlenfalvay and Linderman, 1992).

5.3.5. Activation of plant defense mechanisms

The activation of specific plant defense mechanisms as a response to AM colonization is an obvious basis for protective capacity of AM fungi. The elicitation by an AM fungi of specific plant defense reactions could predispose the plant to an early response to attack by a root pathogen (Gianinazzi-Pearson *et al.,* 1994). The influence of P nutrition on membrane permeability in root cells, concentration, exudation of amino acids and reducing sugars has been proposed as a mechanism regulating mycorrhizal root penetration and colonization (Ratnayake *et al.,* 1978). Root colonization may effect qualitative and quantitative changes in root exudates to alter rhizosphere or rhizoplane microorganisms (Linderman, 1985). Tomato roots inoculated with *Glomus fasciculatum* had increased concentrations of phenylalanine and serine; these two amino acids being inhibitory to root-knot nematode development. Higher amounts of catechols, which inhibit *Sclerotium rolfsii* growth *in vitro,* have been reported in mycorrhizal roots (Krishna and Bagyaraj, 1986). Benhamou *et al.* (1994) reported that production of unusual material in plants infected with *Fusarium oxysporum* f. sp. *chrysanthemi* whether colonized with AMF or not and coating of most of the intercellular spaces with similar substances was the most typical host reaction. Such deposits may be infused with phenolics as the deposited material often interacted physically with the walls of involving hyphae exhibiting morphological and cytological changes. These deposits, in addition to acting as a barrier to fungal spread, also display fungitoxic activity. A few electron-opaque structures resembling the deposits were found in some cells and intercellular spaces of non-infected mycorrhizal

carrot roots but were absent in infected, non-mycorrhizal carrot roots. AM fungi resulted in increased concentration of antifungal chitinase, arginine accumulations in roots which suppresses sporulation in *Thielaviopsis* (Baltruschat and Schönbeck, 1975; Dehne *et al.,* 1978). Morandi *et al.* (1984) found increased concentrations of phytoalexin-like isoflavonoid compounds in AM roots compared to those in non-AM soybean. They postulated that such materials could account for the increased resistance to fungal and nematode root pathogens of AM plants compared to non-AM plants. Enhanced accumulation of coumestrol in soybean helps explain how mycorrhizal infection decreases the development of pathogenic nematodes more consistently than that of fungi (Hussey and Roncadori, 1982; Kellam and Schenck, 1980). Suresh and Bagyaraj (1984) reported that AM inoculations reduced root-knot infestations and such plants have increased quantities of sugars and amino acids, which play a role in suppressing nematode reproduction. Pre-inoculation of tomato roots with *G. fasciculatum* coupled with such biochemical changes as increased amount of lignins and phenols made tomato resistant to the root-knot nematode *Meloidogyne incognita*.

Cordier *et al.* (1998) showed that the induction of resistance to *Phytophthora parasitica* in mycorrhizal tomato plants is governed by the cellular and molecular phenomena underlying bio-protections, using a spilt root experimental systems, they showed that the control of *P. parasitica* in mycorrhizal tomato root systems involves induction of localized resistance in arbuscules containing cells and systemic resistance in non-mycorrhizal tissues. Ultrastructural investigations coupled with histochemical immunocytochemical analyses have provided evidence that decreased pathogen development in both mycorrhizal and non-mycorrhizal parts of mycorrhizal root systems is associated with modifications in host cells, together with the accumulation of defence-related molecules. Further investigations were aimed at characterizing plant genes expressed during the bioprotection of mycorrhizal tomato infected with *P. parasitica*. The induction of defence-related enzymes like hydrolytic enzymes chitinase, chitosanase and β - 1,3 - glucanase in mycorrhizal tomato roots against *Phytophthora parasitica* was also reported by Pozo *et. al.* (1998).

5.4   Integrated disease management strategies

In order to ensure compatible combinations of AMF and antagonists which, occur in the same soil, growers should inoculate the seeds with appropriate cultures and avoid chemical pesticides. Saleh and Sikora (1989) showed that the fungicides such as benomyl and carbendazim significantly reduced the antagonistic activity of *G. fasciculatum* that affects reproduction in *M. incognita* on cotton when applied as a drench at planting time. The number of nematodes increased significantly on mycorrhizal plants when benomyl was

applied at planting time but no significant change was noticed with similar applications of carbendazim, which is less toxic to *G. fasciculatum* than benomyl. However, when the fungicides were applied 25 days after planting, there were fewer nematodes in mycorrhizal plants. Neither of the fungicides altered nematode populations on non-mycorrhizal plants. Similarly, *G. fasciculatum* did not affect nematode populations density when applied 30 days after or simultaneous inoculations at planting time but when it was applied 50 days later, it reduced the nematode population significantly. Co-inoculation efficacy of mycorrhizae with other biocontrol agents needs to be optimized for both pot culture and field conditions.

5.4.1. Host-symbiont-nutrient status
Growth responses to AM vary depending on the mycorrhizal dependency of the host, the endophyte strain, and the soil fertility level (Harley and Smith, 1983). Preliminary research has been done to determine whether a relationship is dependent on an AM fungal species or mixture of species that are indigenous to an area and stimulate plant growth at moderately fertile soils. This is necessary for assessing, to a closer approximation, the response of mycorrhizal plants to pathogen infection for the soil type and fertility level occurring in the field. A better approach would be to use varying soil P levels that encompass field recommendations to produce non-AM plants of equivalent size and similar P status as mycorrhizal plants. These conditions are necessary to find out the real mechanism of the tolerance to pathogen attack shown by AM plants.

5.4.2. Statistical models for mycorrhizae-pathogen interactions
Factorial regression analyses that evaluate the response of qualitative treatments (mycorrhizal versus non-mycorrhizal plants receiving a particular P regime) over varying quantitative treatments (Pathogen Pi) are essential for valid statistical interpretation of data. In addition, frequency values of nematode cycles over physiological time have been used to compare the effects of AM fungi and P nutrition on nematode parasitism (Smith *et al.*, 1986). Another quantitative approach adaptable to mycorrhiza - nematode interactions is the Seinhorst damage functions, $Y = m + (1-m)Z^{P-T}$, to determine if the model parameters T (tolerance limit), m (minimum yield), or Z (nematode virulence) are affected differently in mycorrhizal and non-mycorrhizal P-fertilized plants (Wallace, 1983).

5.4.3. Inoculum density and inoculation strategy
A high pathogen inoculum density can overwhelm biocontrol agents (Cook and Baker, 1982) and this has been shown in AM studies as well (Schenck, 1987). It is difficult to draw firm conclusions about the potential for biocontrol unless a range of pathogen inoculum densities is used. While varying the inoculum densities of pathogen to produce different levels of disease incidence or yield reduction,

however, one should avoid the use of a Pi (inoculum density) that so severally stunts or kills plants that AM fungi are not given an opportunity to colonize roots and stimulate growth (MacGuidwin *et al.,* 1985).

Because root infections by fungal and nematode pathogens precede mycorrhizal root colonization (Linderman, 1985) the sequence in which plants are inoculated may affect the nature of the interaction (Smith, 1987). Thus, an inoculation method used in many studies has been to inoculate plants with the AM fungus 2 - 4 weeks before inoculating them with the pathogen (Cooper and Grandison, 1986; Smith *et al.,* 1986). This technique allows AM fungi enough time to colonize roots before they are challenged by the pathogen, however, it is restricted to containerized or transplanted hosts (Hussey and Roncadori, 1982). Since, AM fungi are generally considered slow colonizers, it seems logical that a minimum level of AM fungal root colonization is required for the fungus, to have any effect on the pathogen. AM fungal inoculum dose of 0.5 - 5.0 spores per gram of soil has been used to produce optimal growth responses and maximum root colonization levels. Although such thresholds of the levels of colonization required to affect nematode activities have been reported (Grandison and Cooper 1986; Smith *et al.,* 1986), high levels of AM fungal root colonization have not reduced the degree of root infections by fungal pathogens (Davis and Menge, 1980, Graham and Menge, 1982).

6.   Inoculum production and delivery of AM fungi

Since AM fungi are obligate symbionts, they are always produced on roots (Mukerji *et al.,* 2002). The method of culturing and inoculum production of AM fungi vary from pot culture techniques of Mosse and Gerdemann (Wood, 1985) to currently used techniques such as on farm production (Sieverding and Barea, 1991), nutrient film technique (Mosse and Thompson, 1984), aeroponics (Jarstfer and Sylvia, 1995), and axenic culture (Fortin, 2002). Apart from the host plant (Sreenivasa and Bagyaraj, 1988), many factors such as temperature (Furlan and Fortin, 1973), light (Ferguson and Menge, 1982), pot size and soil fertility (Menge *et al*., 1978), the particle size of the growth substrate (Gaur and Adholeya, 2000b) are known to affects inoculum production of AM fungi.

6.1   On-farm production of AM fungi

On-farm inoculum production is a promising technique for large-scale AM fungal inoculum production where the inoculum is produced on-

farm, directly on the site of its application using local resources. On-farm inoculum production, therefore, is a technique where the fungal inoculum is produced *in situ*, in the farmers own nursery. An essential component of such technique is that besides producing higher plant yield from such cultivation by farmers, this technique also supports mass production of highly efficient inocula. The mycorrhizal inocula can then be prepared by harvesting roots of growing plants and applied in rest of the field over a period of time. The soil left in the nursery after removing the roots also contain large amounts of AM fungal propagules which serves as the source for further and continued production of inocula for in-house use for the farmer. Gaur and Adholeya (2002) have conducted experiments in marginal soil for enhancing crop production along with producing a higher number of AM fungal propagules. The procedure is described in detail by Sieverding and Barea (1991) and can produce 5000 L of soil inocula from a 25 m² plot

Gaur and Adholeya (2002) reported production of five fodder crops, *Zea mays*, *Medicago sativa*, *Trifolium alexandrinum*, *Avena sativa,* and *Sorghum vulgare* in marginal soil along with producing a high number of indigenous AM propagules. Mycorrhizal inoculation increased yield in terms of shoot dry weight by 257% in *T. alexandrinum* followed by 50% in *A. sativa,* 28% in *Z. mays,* 20% in *M. sativa* and 6% in *S. vulgare*. Plant species showed high root infection. Spore production and infectious propagules (IP) were as high as 78 spores/IP g $^{-1}$ and 103 spores/IP g -1 in *S. vulgare.* In another study (Gaur and Adholeya, 2000a), A mixed culture of indigenous endomycorrhizal fungi was multiplied in a nursery and tested for its ability to promote growth and yield of three agricultural crops. Inoculation response in terms of yield increase was maximum in onion (70%) whereas garlic and potato showed 30% and 48% increases respectively. Colonization in onion reached approximately 85% of root length, followed by garlic (70%) and potato (65%). In addition, Gaur *et al.* (2000), demonstrated the potential of three crops coriander, fenugreek and carrot as potential host for on farm inoculum production.

6.1.1 Procedure for on-farm inoculum production of AMF

Starter culture: Large-scale production of AM fungi begins with a starter culture. The starter culture can be procured either by isolating or by ordering it from various laboratories that maintain pure cultures of specific interest.

Disinfection of soil: Soil in nursery beds should be sterilized either with methyl bromide or formalin by drenching it up to at least 18 inches depth with either of the solutions. In one of the study conducted at Forest Research Institute, Dehradun, it was found that sunlight is equally effective for soil sterilization. After treating with chemicals the soil should be covered for 3 days and then kept open for at least 8 days before commencing any operation. Sun light for sterilization, involves covering the soil with transparent polythene sheets for a minimum of 20 days (Mukerji and Garg, 1988a).

Preparation of nursery bed: The soil of nursery should be raised up to 30 cm. This can be done making surrounding furrows of the similar depth. Soil should be thoroughly mixed and preferably sieved. If the soil is compact, sand may be mixed for good mycorrhizal development in a ratio of 2:1.

Sowing and inoculation: Furrows of 6 cm depth can be made in the nursery beds and AM propagules, mixed with any suitable carrier placed in the furrows. The inoculum should contain at least 30 - 40 spores per gram of substrate. The inoculum should be covered with a thin layer of soil on which host seeds preferably monocots should be sown.

Maintenance and monitoring: The beds should be watered when required and should be kept free from weeds. After 3 months, the extent of colonization and spore production could be assessed.

6.2 Traditional culture methods

The most frequently used technique for increasing propagule number has been the propagation of AM fungi on a suitable host in disinfested soil using pot cultures. Examples of the plants that have been used successfully include alfalfa, maize, onion, sudan grass. Generally, the characteristics of an efficient host include rapid and high root colonization, faster roots growth, and greater number of root hairs. Hosts can be propagated from seeds that may be disinfested with Sodium hypochloride or hydrogen peroxide. Hepper (1984) reviewed procedures for disinfestation and for germinating spores. William (1990) mentioned detailed methods for reducing contamination of colonized root pieces. The most effective methods use chlorine compounds, surfactants and a combination of antibacterial agents. We routinely decontaminate spores by incubating in 2% chloramine T, 200 ppm of streptomycin sulfate for 15 min followed by 4-5 rinses in distilled water.

All the components of the culture system are disinfected before initiation of pot culture. The objective here is to kill the existing AM species, pathogenic organisms and weed seeds. The most commonly used method is the heat pasteurization where large batches of soil may be treated by heating to 85 ºC for two 8-h periods with 48 hr between treatments in a commercial soil pasteurizer.

Conducive environmental conditions for culturing of AM fungi are a balance of light intensity, adequate moisture, and moderate temperature without detrimental addition of fertilizers or pesticides (Jarstfer and Sylvia, 1992). Good light quality and high photosynthetic flux density are necessary for high root colonization and spore production (Bereau *et al.,* 2000; Nagahashi *et al.,* 2000; Whitbeck, 2001). Where natural conditions are poor, supplemental high density lamps can be used. Soil moisture effects AM fungal development directly or indirectly (Al-Karaki *et al.,* 1998; Jacobson, 1997). Excessive soil moisture can encourage the growth of hyperparasites on spores in culture. In addition, excessive moisture will create anaerobic conditions, which may be detrimental for AM fungal development. Indirectly, any moisture conditions that inhibit primary root growth will reduce the development of mycorrhizal colonization. The best strategy is to apply water regularly to well drained substrate. Similarly, soil temperature is also important for the fungus and indirectly as it affects the host chosen (Braunberger *et al.,* 1997; Vogelzang *et al.,* 1993). Sporulation is positively correlated with soil temperature from 15 to near 30 ºC for many AM fungi, however at higher temperature, sporulation is decreased as the host is stressed. Care should be taken that moisture and temperature conditions that prove best for the host should be selected for AM fungal multiplication.

Amendments with fertilizers and chemicals can have both beneficial and detrimental effects on the development of colonized root systems and sporulation. Response to P and N fertilization may be strain dependent (Douds and Schenck, 1990) and are effected by relative amounts of N and P. Nutrients can be applied using one of these three approaches: i) any balanced nutrients except P, which can be applied up to 10 fold dilution than recommended; ii) apply dilute but balanced nutrients frequently; iii) mix a time release fertilizer in the substrate.

In greenhouse, specific isolates of AM fungi should be kept well separated from each other. To initiate pot cultures, a layer of inoculum is placed 1-2 cm below the seed or cuttings. Inoculum may consist of spores, colonized root pieces, vesicles and hypha. Initial isolates are obtained by trapping the infested soil collected from the field. However, these mixed cultures should rapidly be processed for purification and single species cultures initiated. Detailed methods for pot culturing and extensive discussion on these methods are provided by Jarstfer and Sylvia (1992) and Sylvia (1994). Cultures reaching high propagule density (10 spores per gram) after a number of multiplication cycles can be stored using suitable methods (Kuszala *et al.,* 2001; Staddon *et al.,* 2001) after air drying.

Furthermore, AM fungi have been cultured with plant host in different substrates such as sand, peat, expanded clay, perlite, vermiculite, soilrite (Mallesha *et al.,* 1992), rockwool (Heinzemann and Weritz, 1990) and glass beads (Redecker *et al.,* 1995). Culturing AM fungi in soilless media avoids the detrimental organisms and allows control over many of the physical and chemical characteristics of the growth medium. These media are more uniform in composition, weigh less, and provide aeration better than the soil.

6.3   AM fungal culture using aeroponic and hydroponic culture

The major benefit of aeroponic and hydroponic culture systems is that colonized roots and spores are produced free of any substrate, permitting more efficient production and distribution of inocula. Here, plants are inoculated with AM fungi and grown in sand or vermiculite for 4-5 weeks under conditions conducive for rapid colonization, after which they are washed and non-destructively checked for colonization and then they are transferred into the system (Mukerji *et al.*, 2002). Aeroponics is that branch of soilless culture that deals with no rooting substrate other than the air in which the roots are suspended. The principle is to grow plants with their root system exposed constantly to an aerated mist of dilute nutrient solution. Unlike soil culture, hydroponics or other traditional growth cultures, aeroponics show good root hair development due to highly aerated environment surrounding the root system. Aeroponic culture allows control of

root zone temperature, nutrition, moisture, and gaseous phase. Aeroponic system for the production of arbuscular mycorrhizal fungi was first used by Sylvia and Hubbell (1986). The system was adopted for mycorrhiza production by the utilization of seedlings with roots pre-colonized by a AM fungus and the use of modified Hoagland's nutrition (1950) with a very low phosphorus level. In the aeroponic system, colonization and sporulation was superior to that reported in soil-based pot culture. Uniform colonization (>75%) of *Paspalum notatum* roots by *Glomus mosseae* and abundant sporulation (5 spores per cm colonized root) were obtained after 8 weeks in aeroponic culture. Colonization of roots by *G. intraradices* reached 50% after 12 week and a mean of 8 spores per cm of colonized root were observed. Sylvia and Jarstfer (1992) further determined viability and density of aeroponically produced inocula after shearing. Root samples were harvested from the aeroponic culture blotted dry, cut into 1-cm pieces, and sheared in a food processor for up to 80s. Shearing aeroponically produced inocula reduced particle size. Propagule density increased with decreasing six fraction down to size of 63 mm, after which propagule density decreased. The weighed average propagule density of the inoculum was 135, 380 propagules per g (dry weight) of sheared root material. Aeroponic root inoculum was stored dry at 4°C for 23 months without significant reduction in propagule density. *Entrophospora kentinensis* was successfully propagated with bahia grass and sweet potato in an aeroponic system by Wu *et al.* (1995). Spores were produced 6 weeks after the host plants were transferred to an aeroponic chamber. Mohammad *et al.* (2000) reported the production of *Glomus intraradices* in an aeroponic system where they compared the conventional atomizing disc with the ultrasonic nebulizer technology as misting sources. Growth of pre-colonized arbuscular mycorrhizal (AM) roots of Sudan grass was achieved in both chambers used but both root growth and mycorrhization were significantly faster and more extensive in the ultrasonic nebulizer system than in the atomizing disc system. Shearing of the AM fungi (AMF) infected roots in both the systems did not reduce inoculum viability, as evident from the MPN data. However, sheared roots from the ultrasonic nebulizer system had significantly

more infective propagules than those produced in the atomizing disc system.

Hydroponics or Nutrient film technique was adapted for AM fungus inoculum production by Mosse and Thompson (1984). Host plants to be cultured are placed on an inclined tray over which flows a layer of nutrient solution. As in the aeroponic culture, seedlings must be precolonized in another media. Elmes and Mosse (1984) described experiments with maize and other hosts in nutrient flow culture for enhancing AM fungal inoculum production. Dugassa *et al* (1995) presented a hydroponic system for culturing and maintaining the VAM fungus *Glomus intraradices* in symbiosis with linseed (*Linum usitatissimum* L.) under greenhouse conditions in pure nutrient solution. They obtained large quantities of mycorrhizal host plant roots as well as extramatrical mycelium and chlamydospores free of impeding residues of solid substrate components. Starting from linseed donor plants inoculated in sand and transferred to the nutrient solution, new infections arose within the fast growing root system, hyphae spread out into the liquid and infected mycorrhiza-free receptor plants

6.4 Monoaxenic Culture of AM fungi

Recently, Ri T-DNA-transformed roots have been used to obtain colonized root cultures. Becard and Fortin (1988) presented a detail evaluation of the root organ culture technique and reported basic improvements necessary for VAM fungus colonization of roots. Cultures are initiated by transfer of pregerminated, surface sterilized spores or surface sterilized, colonized root pieces into petriplates of minimal media (Becard and Fortin, 1988) or modified Strullu-Romand medium (Declerck *et al.,* 1996).

Douds (2002) presented monoxenic culture of *Glomus intraradices* Schenck and Smith with Ri T-DNA transformed roots in two-compartment Petri dishes as a very useful technique for physiological studies and the production of clean fungal tissues. Experiments were conducted to increase the efficiency of this method for the production of arbuscular mycorrhizal fungus spores. Approximately 20,000 spores could be harvested every 2 months from the distal (fungus only) compartment of a 9-cm-diameter divided Petri

dish.   The method requires replacement of the gelled media in the distal compartment and resupply of 200 mg glucose to the proximal (root) compartment coincident with harvest of spores.   These modifications resulted in an approximate threefold increase in spore production per unit time over the standard split-plate culture technique. Plenchette *et al.* (1996) reported *Glomus versiforme* associated *in vitro* with Ri-T-DNA-transformed carrot root and after 4 months of cultivation, numerous axenic arbuscular mycorrhizal (AM) propagules were obtained.   Three successive generations of spores and mycorrhizal root pieces were obtained by re-associating a 4-month-old root piece with a new carrot root.   A biological test was also conducted to assess the infectivity of the three generations of inoculum.

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