PART I

Chapter 1

GENERAL INTRODUCTION
Plant pathology has now entered into the experimental phase, where physiology, bio-chemistry and other allied branches have come to play an important role in elucidating the physiology of disease. During the past quarter century a large number of workers like Bateman (1963, 1969), Wood (1967), Albersheim et al. (1969), Byrde and Cutting (1971) and Dimond (1956, 1971) have immensely contributed to advance the knowledge of the mechanism of disease development. Disease is recognized now as a manifestation of several complex interactions going on between the host and the parasite.

Diseased plants are distinguished by changes in their structure or physiological processes which are brought about by unfavourable environment and/or by one or another parasitic agency. There is no clear defined line of distinction between normal or healthy plants and abnormal or diseased plants. Plant disease therefore, is like many biological phenomena difficult to circumscribe or define (Walker, 1969). We may, however, think of disease plants as those which have become
altered in their physiological and morphological development to such a degree that signs of such effects are obvious. Several workers have carried out biochemical and physiological investigations on various pathogens causing plant diseases. These studies include enzymology, pathogenesis, and respiration of pathogenic fungi.

The term respiration was first used by animal physiologists to describe the breathing movements of animals, but was subsequently extended to include the chemical reactions by which the complex organic substances of a cell are broken down to release carbon dioxide, water and energy. The elucidation of the nature of these reactions and the pathway of oxidation-reduction reactions in the cell has been one of the most exciting developments of the biochemistry and physiology in the past few decades (Giese, 1973).

All the living organisms require a regular supply of kinetic or active energy for various kinds of vital activities. For this they keep energy stored in various organic substances, especially the carbohydrates, fats and proteins. Cell foods are the sources of all the building material and energy available to the organism. Respiration is essentially a process of oxidation and decomposition of organic compounds particularly simple carbohydrates, in the living cells with the release of energy. By this process the potential or chemical energy stored in the organic compounds is released in a step-wise
manner in the form of active or kinetic energy, under the influence of enzymes, and is partly made available to the protoplast for its manifold vital activities. The physiological activities of the cell are the consequence of the operation of a physio-chemical system.

Respiration occurs generally in all the living cells. Carbohydrates, mainly the hexose sugars, act as the substrates which are oxidized in the cells. After the consumption of the carbohydrates the fats and lastly the proteins form the respiratory substrate. When fats serve as the substrate in plants, they must first be hydrolysed to fatty acids and glycerol before oxidation can proceed. In case of proteins they are first hydrolysed to amino acids, which are then oxidized. Thus the phenomenon of liberation of energy from organic substances in a catabolic process is described as respiration. It is also called as "cellular respiration" because it occurs only in the living protoplasts. The most frequently occurring form of cellular respiration is aerobic oxidation of glucose, which represents two broad phases: (1) glycolysis - or the conversion of glucose to pyruvic acid (anaerobic oxidation of glucose) and (2) the citric acid cycle, also known as the tricarboxylic acid cycle (TCA cycle), which further oxidizes pyruvic acid to carbon dioxide and water, utilizing oxygen as the final hydrogen acceptor.

The most common glycolytic pathway is the Embden-Meyerhof
(EM) pathway named after the two pioneer investigators. However, alternate pathways also exist. One of the most important of these being the hexose monophosphate (HMP) pathway which may account for as much as ten per cent of glucose catabolism (Cheldelin et al., 1962 and Lehninger, 1970). This pathway was elucidated by Hovecker Racker and their collaborators (Racker, 1954; Hovecker and Mehler, 1955; Hovecker 1962).

The HMP pathway is known under a wide variety of names, such as, the hexose monophosphate oxidative shunt, the Warburg-Lipmann-Dickens pathway, the pentose phosphate cycle, the direct oxidative pathway, and the phosphogluconate oxidative pathway. The other glycolytic pathways are the Entner-Doudoroff (ED) pathway and glucuronate-xylulose pathway.

The respiratory metabolism in fungi has been comprehensively reviewed by Blumenthal (1965) and Niederpruem (1965). The EM and HMP pathways have been reported very commonly in a number of fungi, whereas, the ED pathway was recognised conclusively only in the hyphae of Caldarionycen fumago (Ramchandran and Gottlieb, 1963) and in spores of Tilletia caries (Newburg and Cheldelin, 1958). The presence of glucuronate-xylulose pathway in fungi has only been indirectly evidenced by Sastry and Sarma (1957) in case of Aspergillus niger.

Investigations carried out during the past few decades on physiology of fungi have contributed much to the understanding of fungal behaviour.
Metabolic inhibitors and specific respiratory poisons have been extensively used for elucidation of the mechanism of oxidative metabolism in fungi, as it has been proved to be the most fertile technique for various metabolic investigations (Niederpruem, 1965). The occurrence of an active tricarboxylic acid cycle among fungi such as Aspergillus and Penicillium was long suspected, however, its presence was not confirmed. The evidence for the existence of TCA cycle in fungi came from the nutritional studies of Barnett and Bornberg (1960). A further evidence of an operational TCA cycle in fungi came from the studies involving the mode of action of iodoacetic acid on Neurospora crassa in which the respiration and growth were inhibited appreciably by physiological levels of iodoacetate (Ryan et al., 1944). Subsequently, certain mutants of N. crassa were found to utilize various growth substrates associated with the TCA cycle (Lewis, 1949). After considerable efforts involving refinement of existing techniques a functional TCA cycle has been found to be widespread among the fungi.

Reilly and Gottlieb (1974) while investigating the respiratory physiology of Myrothecium verrucaria reported that almost all enzymes of the EMP pathway, the hexose monophosphate shunt, and TCA cycle were active in the cell-free preparations of the mycelium of this organism.

Mathre and Ravencroft (1966) indicated the presence of
a functioning oxidative phosphorylation system in the endoconidia of *Thialaviopsis basicola*, since 2,4-dinitrophenol (DNP) and sodium azide, both stimulated oxygen uptake. These compounds are known to uncouple oxidative phosphorylation.

There has been an increasing use of certain antibiotics as they are found to have specific effect on the enzymes of respiratory metabolism. For example, the electron transport system is affected by antimycin A. Antimycin A inhibits the transfer of electrons from cytochrome b or coenzyme Q to cytochrome c (Keilin and Hartree, 1955; Ramachandran and Gottlieb, 1961; Lester and Fleischer, 1961). Boulter and Derbyshire (1957) demonstrated the general occurrence of cytochromes in 45 fungal species. Besides there are numerous other reports (Darby and Goddard, 1950b; Sussman and Market, 1953; Sih et al., 1955; and White and Ledingham, 1961), of characteristic cytochrome oxidase activity in various fungi. Kikuchi and Barron (1959) and Niederpruem and Hackett (1961) showed that *Fusarium oxysporum* f.sp. *lilii* and *Schizopyllum commune* contained various components of the electron-transport system. Similarly, Dowler et al. (1963) have also reported the presence of electron transport system in terminal respiration of the cell free extracts of *Aspergillus fumigatus, Claviceps purpurea, Gibberella zeae, Glomerella ungulata, Phycomyces nitsus, Pythium debaryanum, Rhizoctonia sp., S. commune, Monilinia fructicola* and *Stemphylium solani* where
antimycin-sensitive site and cytochrome oxidase were reported to be present. Tape et al. (1960) reported that antibiotic nystatin inhibited oxygen uptake of spores of *Monilinia fructicola* and *Botrytis* sp. and suggested that the site of inhibition lies in Krebs cycle.

The antibiotic cerulenin interferes with fatty acid biosynthesis in a wide range of organisms including bacteria, cellular moulds, yeasts and mycelial fungi (Amura, 1976). In some cases cerulenin simultaneously inhibits sterol biosynthesis (John et al., 1974). This antibiotic provides a novel, useful means of examining the general role of lipid synthesis (and presumably membrane lipid synthesis) in the biogenesis and function of cellular membranes and organelles (Brambl et al., 1977).

Brambl (1977) found that mitochondria from dormant spores of the fungus *Botryodiplodia theobromae* did not contain extractable cytochrome c oxidase activity, however, this enzyme activity was elaborated rapidly after 150 minutes of the 240-minutes germination sequence. The elaboration of cytochrome c oxidase activity in germinating spores was abolished by cycloheximide (Brambl, 1977). *Neurospora crassa* was found to be dependent upon the function of the cytochrome mediated electron transport pathway (Stade and Brambl, 1951). The dormant spores contained all of the cytochrome components and a catalytically active cytochrome c oxidase required for
the activity of the standard respiratory pathway, and these preserved components were responsible for the accelerating rates of oxygen uptake which began immediately upon suspension of the spores in an incubation medium. In many cases, the fungal spore germination has been used as an experimental system for study of the physiological mechanism involved in the developmental transition from a state of dormancy to that of active metabolism and rapid growth (Bennet and Brambl, 1983).

Respiratory responses of Helminthosporium sativum, Alternaria solani, Fusarium oxysporum and Colletotrichum capsici induced by four new polyene antibiotic formulations isolated from four Streptomyces sp. were investigated by Belayan et al. (1980). Significant inhibitory effect on endogenous mycelial respiration of A. solani, F. oxysporum, and C. capsici was brought about by tetraene BG 6 and DG 15 polyenes. From these results it was concluded that the mechanism of their antifungal action probably involves their influence on the oxidative metabolism of these organisms.

The fungitoxicity of some fungicides has been attributed to their potentiality for disrupting or blocking certain respiratory enzymes of the fungi (Sisler and Cox, 1960; Tolmsoff, 1962). Horsfall (1956) has pointed out that fungicides can disrupt the smooth turning Krebs cycle at any point. Similar results, showing the interference of fungicides with aerobic respiration have been reported by Allen and
Gottlieb (1970). As yet, the effect of only a few fungicides has been measured on oxidative metabolism of phytopathogens (Sieler and Cox, 1954; Walker, 1955; Tolba and Selma, 1962; Lyda and Burnett, 1970).

Sieler and Cox (1954) reported suppression in the rate of oxygen uptake in conidia of *Fusarium roseum* by thiram. Walker (1955) obtained similar results in conidia of *M. verrucaria*. Carboxin was inhibitory to growth as well as respiration against *Rhizoctonia solani* and *Ustilago maydis* (Mathre, 1970; Ragdale and Sieler, 1970).

Tolba and Selma (1962) while studying the respiration of *R. solani* and *Fusarium culmorum* have reported that mercuric chloride caused a marked fall in respiratory rates. Similarly, Vyas and Saksena (1973) have also reported a strong inhibitory effect of this compound upon mycelial respiration of *Sclerotium rolfsii*.

TMTD and its close relative antabuse (tetra ethyl thiurum disulphide) are known to inhibit metabolism in many other organisms having functional glycolytic pathway, viz., *M. verrucaria* (Walker, 1955), *C. capsici* and *R. solani* (Saksena et al., 1975). Saraf and Soni (1983) found that *A. theobromae* could be adapted to grow on medium having six times the lethal concentration of TMTD. However, the respiratory activity in the adapted fungus was found to be significantly low.

Endogenous respiratory metabolism of various microorganisms, particularly bacteria and yeast has received considerable attention (Dawes and Ribbons, 1962). Besides the yeast, the respiratory metabolism of some fungi have been investigated including \textit{I. carica} (Newburgh et al., 1955), \textit{Penicillium chrysogenum} (Blumenthal et al., 1957), \textit{Allomyces macrogynous} (Bonner and Machlis, 1957), \textit{N. crassa} (Blumenthal, 1963), \textit{Fusarium solani} (Cochrane et al., 1963(1),(2)), \textit{Puccinia

The determination whether a substrate is oxidized or not, at a rate higher than that of the endogenous rate, is very useful in identifying possible metabolic pathways of respiration. The respiration of whole cells and extracts is usually measureable in terms of gas exchange, oxygen uptake or carbon dioxide formation. The basic instrument used in most laboratories is Warburg's constant volume respirimeter described in the manual of Umbreit et al. (1964). The manometric procedure for measuring oxygen utilization has been successfully applied in the studies on a number of fungal forms.

Determination of respiratory metabolism in pathogenic fungi is obviously very important for understanding the physiological and biological aspects of pathogenesis. It is now fairly well admitted that the causation of disease is a complex series of interconnected events impinging upon many vital processes of the host. Evidently the studies of various factors on the metabolic processes are bound to effect the interactions between host and parasite which is often evidenced
through various ways. Moreover, if a pathogen is found to have respiratory pattern other than conventional and universally present ones, then this difference may be exploited for the design of some specific chemotherapeutic agent. Similarly a number of antibiotics, fungicides and certain amino acids have been reported to act as toxicants, by blocking or disrupting the respiratory metabolism in fungi. These may prove useful for devising effective control measures against certain plant diseases.

In the present investigation, an attempt has been made to study the oxidative metabolism of three plant pathogens, viz., *Alternaria alternata*, *Urocera spicifera* and *Schizophyllum commune*. A review of literature reveals that this aspect of study has not been explored so far for these organisms.

*A. alternata* is a well known plant pathogen. It has been reported from a number of hosts (Bilgrami *et al.*, 1979, 1981). Recently Srivastava and Gupta (1963) have reported it to be a severe foliar pathogen of *Zinnia*. Similarly Singh and Suhag (1963) have reported *A. alternata* as causing leaf and pod blight of radish. *D. spicifera* has been mainly found to infect plants of the Gramineae family and has been reported on a number of other host by Bilgrami and his associates (1979, 1981). *S. commune*, a member of Basidiomycetes is a well known wood decaying fungus (Fergus, 1960).
S. commune was previously regarded saprophytic in nature, but later various workers have found its tendency towards parasitic mode of life. Chaudhari and Johar (1931) reported it growing as a parasite on mango and sisam trees. Thereafter it has been reported by various workers growing on logs of timber, tree trunks and branches (Bose, 1946; Bagchee and Bakshi, 1950). Saksena and Vyas (1964) collected it growing on wood and basal parts of living trees of Butea monosperma. Besides this (Bilgrami et al., 1979, 1981) it has also been reported growing on Shorea robusta, Bambusa arundinacea, Saccharum officinarum, Dalbergia latifolia, Hardwickia pinnata, Pinus longifolia, Terminalia mariocarpe. During these studies, in addition to observing the effect of different chemical compounds on respiration, their effects on fungal growth were also observed, so as to record correlation between the two processes if any, on the test pathogens. In order to standardize the methods, the effect of age of culture, starvation period and H-ion concentration on respiration of these organisms were also determined.

The format of the thesis has been planned as follows:

PART I

This part deals with the isolation, purification, general morphological observations of the test fungi and pathogenicity tests. It also includes the description of the procedure adopted for growth studies, spore germination, respirometric
studies, respiratory quotient, quantitative estimation of carbon compounds.

PART II

This part includes the study of the effect of the age of culture, starvation period, and K-ion concentration on mycelial respiration and growth. It also includes studies on respiratory quotient and quantitative estimation of carbon compounds in the mycelia.

PART III

This part deals with the investigations of the effect of carbohydrates, nitrogen sources, metabolic inhibitors, antibiotics and fungicides on mycelial respiration and growth of the test pathogens.

PART IV

It includes studies on spore germination, spore respiration, respiratory quotient, quantitative estimation and effects of various substances on spore respiration of the dormant and germinated spores of two test fungi.

PART V

This part deals with the general summary, conclusions and bibliography.