CHAPTER - I

INTRODUCTION

The colonisation of vascular system of infected plants by pathogens represents a unique adaptation. Until the advanced stages of the disease syndrome, the pathogen is almost exclusively confined to the xylem vessels. Although the fungal mycelium is in contact with the cell wall, the hydrolysis of cell wall polysaccharides and utilization of their break down products varies with the pathogen.

Almost from the beginning of the studies with vascular wilt diseases attention has centered on the causes of wilting. Cell wall degrading enzymes (CWDE) produced by the pathogen/host degrade pectic and other polysaccharides of cell wall and also weaken pit membranes. This results in leakage of materials from xylem parenchyma into the xylem vessels and the collapse of vessels. The release of monosaccharides may have an impact on the growth of pathogen as well as on the production of phytotoxic substances.

The long discussed question of the cause of symptoms in pathological wilting has tended to polarize into support of either an enzyme or a toxin based wilt mechanism. An understanding of how enzymes alone or in combination with other putative chemicals degrade the host cell wall,
how the pathogen grows in that environment and how their toxins are produced (if at all) and how they act is necessary for the evaluation of their role in pathogenesis and development of the wilt syndrome.

Much of the work done on CWDE of wilt pathogens has been confined to the pectic and cellulolytic enzymes. The recent cell wall model proposed by Albersheim's group (Keegstra et al., 1973) has given a new dimension to the area of research of cell wall degrading enzymes.

In the present study emphasis is made on hemicellulases to evaluate their possible role in pathogenesis. Although most of the chapters discuss the regulation of CWDE synthesis and activity the underlying interest is in understanding the regulatory mechanisms that would decide the events leading to pathogenesis.

An understanding of the mechanisms regulating the synthesis or activity of CWDE and the way nutrients function at biochemical level offers a means of controlling the activity of these organisms. An important aspect of pathogenesis is the control of production of such enzymes (Albersheim et al., 1969).

"When a pathogen" say Bateman and Basham (1976) "confronts a cell wall, it faces a complex barrier composed
of polymers with different chemical linkages that require specific enzymes for their degradation. Pathogens have evolved the means to recognize the chemical structures in plant cell walls and elaborate the appropriate enzymes to dismantle the various cell wall constituents. Thus, the interaction between host and pathogen is initiated by the CWDE produced by the pathogen.

**The cell wall degrading enzymes (CWDE):**

**Pectic enzymes:**

The pectic polymer, D-galacturonan is cleaved by two different enzymatic reaction mechanisms, hydrolysis (by polygalacturonases) and \( \beta \)-elimination or \textit{trans}-elimination (by lyases). The hydrolases and lyases are readily distinguishable by their differing requirements for divalent cations (only lyases are cation dependent), by their difference in pH optima (ca 5.5 for hydrolases and ca 8.5 for lyases) and by their products (only lyase products are 4,5-unsaturated at the non-reducing end which permits specific assays based on their absorbance at 230 nm or reaction in the periodate/thiobarbituric acid assay). Endopectic enzymes randomly attack internal linkage in the polymer, degrading it to a series of oligomers. Exopectic enzymes release reducing sugars from one end of the polymer.
At present, eight chain-splitting pectic enzymes are known which are identified by:

1) mode of bond cleavage—hydrolytic or trans-eliminative,
2) site of action, random or terminal, and
3) preference for substrate—pectin or pectic acid.

A) **Hydrolytic cleavage.**

1. **Random cleavage**
   - a-Pectin, preferred substrate: Endo-PMG (EC 3.2.1.41)
   - b-Pectic acid preferred substrate: Endo-PG (EC 3.2.1.15)

2. **Terminal cleavage**
   - a-Pectin preferred substrate: Exo-PMG
   - b-Pectic acid preferred substrate: Exo-PG (EC 3.2.1.4)

B) **Trans-eliminative bond cleavage**

1. **Random cleavage**
   - a-Pectin preferred substrate: Endo-PL (EC 4.2.2.3)
   - b-Pectic acid preferred substrate: Endo-PAL (EC 4.2.2.1)

2. **Terminal cleavage**
   - a-Pectin preferred substrate: Exo-PL
   - b-Pectic acid preferred substrate: Exo-PAL (EC 4.2.2.2)
Hemicelluloses:

Xyloglucans are the major components of the primary cell wall (21% of primary cell wall); other hemicelluloses like xylans (most abundant non-cellulosic polysaccharide) are present mostly in secondary wall in woody tissues. While xylans are essentially \( \beta, 1-4 \) linked D-xylose chains, xyloglucans contain a cellulosic \( \beta-D \)-glucan backbone to which short side chains of xylose are attached at C6 position of at least one-half of the glucose residues. Galactose and fucose are bonded to the xylose; arabinose is a minor component.

Xylanases:

At present two enzymes are known to be associated with the xylolytic activity of the microorganisms, both of which are hydrolases; endo-xylanase (EC 3.2.1.8), which attacks \( (1-4) - \beta-D \)-xylan at random, giving rise to xylooligosaccharides and exo-xylanase or \( \beta \)-xylosidase (EC 3.2.1.37), cleaving the same substrate from the non-reducing end leading to the production of monosaccharide xylose.

Arabinogalactans consist of a chain of arabinose appended to another chain of galactose and few side chains with single sugar, whose location is not known. Plants contain two structurally distinct types of arabinogalactans;
type I and type II. Those of type I have been described as attached to rhamnogalacturonan chains in pectins. These chains with L-arabinofuranose residues attached in side chains. Type II arabinogalactans are highly branched polysaccharide with ramified chains of β-D-galactopyranose residues mutually joined by 1-3 and 1-6 linkages. These contain small proportion (5%) of galacturonic acid residues. In Albersheim's cell wall model the type II arabinogalactans link the hydroxyproline rich protein component to the rhamnogalacturonan.

Arabinogalactan degrading enzymes (arabanase and galactanase) are assayed by monitoring the release of reducing groups from arabinogalactan and by identifying their reaction products by chromatographic procedures.

The cellulase complex:

Cellulase is a unique system in which several enzymes act in a cooperative sequential manner to carry out the complete hydrolysis of native cellulose to glucose. As recognized today (against C₃ and Cₓ concept of Reese, 1956) the cellulase complex comprises of three enzymes (Mandels, 1982; Montenecourt et al., 1979). These enzymes are: exo-β-glucanase (EC 3.2.1.91), endo-β-glucanase (EC 3.2.1.4) and β-glucosidase (EC 3.2.1.21). Some confusions do exist in the nomenclature of the enzyme which acts on crystalline
cellulose. But by and large it is designated as exoglucanase (formerly C₁).

Much of our understanding of enzyme regulation is derived from prokaryotes, particularly lac operon of E. coli given by Jacob and Monod (1961). It is well known that lac repressor can bind to a specific site on the DNA to cause β-galactosidase repression. In presence of an "inducer" which forms complex with the repressor and liberates it from its complex with DNA and repression is released. The repressor acts at the operator site, and, therefore, mutations in this region prevent binding of repressor and allow constitutive enzyme synthesis from the adjacent structural genes. Constitutive mutants can arise from mutations in the regulator gene also.

Regulation of enzymes in eukaryotes is much complex, due to the structural complexity of genetic apparatus. Even in fungi the complexity of regulation has not provided a basis for a general theory (Burnett, 1975; Fincham and Day, 1971). Many of the systems examined do not confirm the bacterial model. In fungi several enzymes of related function are associated as aggregates rather than acting in sequence as in bacteria (Case and Giles, 1971).

The environment of the pathogen determines the production as well as the type of the enzymes produced.
Catabolic enzymes that attack the exogeneous substrates, as a rule are inducible enzymes whose production is initiated only in presence of specific compounds structurally similar to the substrate or its reaction products. In case of polysaccharidases, polysaccharides per se by their very nature cannot act as inducers, because their molecular size would prevent their entry into the cell, and also most of them are inherently insoluble. In some cases the organism is unable to grow on a particular substrate. It all depends on the extent to which the polymer is degraded into low molecular weight components, on the existence and proper functioning of a carrier molecule and on existence of metabolic pathways to metabolize the compound.

The principal transport system of carbon compounds is of two types: (1) constitutive and (2) inducible. In constitutive enzyme system, the necessary carrier proteins are always present in the cell. In inducible systems, the proteins become functional in presence of the inducers. It is known that inducible enzymes are also usually formed to a low extent in absence of inducers, this is known as basal synthesis. The little amount of enzyme present may be sufficient to act on the substrate and release small fragments which then enter the cell and stimulate more production. Constitutive enzyme synthesis on the other hand does not fluctuate with the presence or absence of
the inducer. The criterion for inducibility is, generally, the detection of greater enzyme activity when a microorganism is grown on a polysaccharide substrate than on a substrate such as glucose (Fuchs, 1965; Keen and Erwin, 1971).

**In vitro Regulation:**

**Pectic enzymes:**

The more studied pectic enzymes with regard to regulation are PAL of *Erwinia chrysanthemi* and *Erwinia carotovora* pv. *carotovora* (Chatterjee et al., 1979; Chatterjee and Ferguson, 1981; Collmer and Bateman, 1982; Moran and Starr, 1969; Zucker and Hankin, 1970).

Chatterjee et al. (1979) reported PAL of *E. chrysanthemi* that accumulates to a several fold higher level during incubation with galacturonan than during incubation with glycerol, gluconate or galacturonic acid. The rate of PAL synthesis is at least 20-fold higher during logarithmic growth on glycerol plus galacturonan, than it is on glycerol alone (Collmer and Bateman, 1979). Since *E. chrysanthemi* increases its rate of PAL production during incubation with galacturonan the bacterium is clearly able to recognize the presence of the polymer in the environment. The strong possibility that the polymer (10,000-20,000 MW) can not be transported suggests that recognition
may be mediated by oligomeric products released from the polymer by basic levels of extracellular pectic enzymes. Such a mechanism is generally assumed to operate for all inducible extracellular enzymes.

Collmer and Bateman (1982) analysed the induction process in E. chrysanthemi strain-630, that apply specifically to the production of extracellular depolymerases. The findings are:

1) If extracellular digestion of the polymer is a rate-limiting step, then induction should be stimulated by supplying oligomeric products.

2) Induction by oligomeric products is likely, if these products, but not the polymer, are assimilated.

3) If induction still occurs, when the polymer is incorporated in an insoluble structure, then mechanism of releasing soluble inducer must be operating.

4) Identification of the actual inducer within the cell may indicate that digestion of the polymer is obligatory for inducer formation.

Studies by using digalacturonic acid (DGA), unsaturated digalacturonic acid (UDGA) and galacturonan reveal that PAL synthesis increases more rapidly and to higher
initial levels in medium containing the dimers (Collmer and Bateman 1982; Ferguson and Chatterjee, 1981). *E. chrysanthemi* the PAL and exo-PG productions are coordinately regulated and the action pattern of these two enzymes appears to be complementary in the degradation of pectic polymer to disaccharide that stimulates pectic enzyme production (Collmer et al., 1982).

*E. carotovora* pv. *carotovora* also produces high amounts of PAL in presence of DGA (Hubbard, 1978; Tsuyumu, 1977). When grown on glycerol or galacturonan medium, *E. carotovora* pv. *carotovora* Ec 1 required some lag period, this could be due to the time necessary for basal enzyme levels to release sufficient inducer from the polymer. However, when incubated with DGA the lag period was shortened (Chatterjee et al., 1979). Monomer galacturonic acid was inferior to dimer or the polymer for production of PAL in *E. carotovora* pv. *carotovora* (Chatterjee et al., 1979; and Tsuyumu, 1977).

Pectic enzymes of fungi are variable in their regulation, even within species e.g. some isolates of cotton strains of *Verticillium albo-atrum* are inducible and others are constitutive for PG synthesis (Keen and Erwin, 1971; Mussel and Strouse, 1972). Pectin esterase production by *F. oxysporum* f. sp. *lycopersici* is constitutive (Waggoner and Dimond, 1955) but it is inducible in f. sp.
vasinfectum (Lakshminarayanan, 1958). Inducible endo-PL has been reported from *F. oxysporum* f. sp. *lycopersici* (Sherwood, 1966; Cooper and Wood, 1975). Mathur (1976) reported inducible pectic enzyme production by *V. albo-atrum*, the organism was unable to produce these enzymes in absence of pectic substances, while *V. dahliae* produced constitutive pectic enzymes on glucose, addition of pectic substances to the glucose containing medium not boost up enzyme production as occurs with the inducible enzymes. PG of *F. oxysporum* f. sp. *lycopersici* is inducible while that of *V. albo-atrum* is constitutive (Mussel, 1972). However, PG of *Pyranoeheata terrestris* is inducible in all 10 isolates from various locations (Horton and Keen, 1966).

Different pectic enzymes of a single isolate may be under different control mechanisms eg. some cotton, hop and potato strains of *V. albo-atrum* produce PG constitutively and PL adaptively (Mussel and Strouse, 1972; Talboys and Bush, 1970) and PMG and PAL of *H. sacchari* (Dube and Bordia, 1983). *F. oxysporum* f. sp. *lycopersici* secretes both PME and PG into the culture medium when pectin serves as carbon source, but it produces only PME when pectin is replaced by glucose. Similar differential control of synthesis of endo- and exo-PGs has been reported for three species of *Botrytis* (Hancock et al., 1964). Later these authors (1966) established differential control of PAL and exo-PG synthesis in *Colletotrichum trifolii*. 
Bateman (1966) reported differential control mechanisms of pectolytic enzyme production in *Fusarium solani* f. sp. *phaseoli*.

Like differential induction, there are reports of Co-ordinated induction in which dual enzyme actions may be associated with a single enzyme fraction even after extensive purification e.g. PME activity remains in a homogeneous PG of *B. cinerea* and in a PAL of *Clostridium multifermentans* (Urbanek et al., 1975; Miller and Mac Millan, 1970). Similar type of observations were made by Wang and Keen (1972), when they failed to remove PAL activity from PG of *V. albo-atrum* and isozymes of PL and PG retained almost identical activity after wide and narrow range isoelectric focussing (Cooper et al., 1976).

Pectic enzymes hydrolyses and lyases have been almost invariably induced by polygalacturonides (Collmer and Bateman, 1982, Dube and Mathur, 1977) and in some cases by the monomer D-galacturonic acid (Cooper and Wood, 1973). These authors working with wilt pathogens *V. albo-atrum* and *F. oxysporum* f. sp. *lycopersici* found uronic acids and sugars to be inducers of various polysaccharidases, if their supply did not exceed 10 μg/ml sugar level in the culture medium. They concluded that each polysaccharidase of the two fungi was induced specifically by the monomer or dimer predominant in the enzymes specific
polymeric substrate. The degree of specificity of their induction was shown to be very high. Both PG and PL were induced by galacturonic acid and not by any other sugar. None of the enzymes were produced above basal levels on the restricted supply of glucose, which is indicative of their inductive nature. Structural derivative of D-galacturonic acid such as L-galacturonic acid, mucic acid, dulcitol and tartronic acid were also found to be inducers of pectic enzymes (Horton and Keen, 1966; Phaff, 1947), which suggests induction by a common metabolite derived from these analogues.

**Cellulases:**

Most of the studies on regulation of cellulases have been done with *Trichoderma* sps. All naturally occurring *Trichoderma* sps. require an inducer for cellulase synthesis. Cooper and Wood (1973) found that in case of *V. albo-atrum* and *F. oxysporum* f. sp. *lycopersici* cellulases were induced by cellobiose but not glucose suggesting their inductive nature. Both *V. albo-atrum* and *V. dahliae* produce inducible cellulases (Mathur and Dube, 1978). Neither of the pathogens could produce the enzymes in absence of cellulose or cellulose derivatives as carbon source. The constitutive production of cellulase is reported by Strider and Winstead (1961) for *Cladosporium cucumerinum* and Winstead and Mc Camb (1961) for *Pythium*
aphanidermatum. Constitutive endo-glucanase synthesis has been reported for *P. solanacearum* (Husain and Kelman, 1962; Podile, 1986), and *E. chrysanthemi* (Garibaldi and Bateman, 1970, Prabakaran, 1984).

The natural inducer of cellulase is unknown, however all the available strains of *T. reesei* and inducible by cellulose cellobiose, lactose, sophorose and repressed by glucose. The usual assumption is that low constitutive levels of cellulase reacts with cellulose to produce a soluble product which enters the cell and induces enzyme synthesis. Cellobiose, the end product of cellulase was shown to be the inducer of cellulase and several instances (Cooper and Wood, 1973; Goodenough and Maw, 1975; Horton and Keen, 1966; Mandels and Reese, 1960; Mathur, 1976). However, cellulase production may not start until the disaccharide is virtually depleted from the culture of *V. albo-atrum* (Gupta and Heale, 1971).

Sophorose a disaccharide, which is not an end product of cellulolysis, is a powerful inducer of cellulase (Mandels and Reese, 1962; Eriksson and Hamp, 1978). It has been shown to be synthesized by transglycosylation activity of *T. viride* cellulase (Toda et al., 1968). Induction by sophorose results in the formation of a complete array of cellulolytic enzymes analogous to 48 h cellulase induction (Sternberg and Mandels, 1980). In most of the cases
cellulase appears in the medium only when most of the sophorose has been taken up, but the enzyme production ceases after depletion of sophorose from the medium. The optimum concentration of sophorose for cellulase synthesis was found to be $10^{-3}$ M while concentration higher than $10^{-1}$ M inhibited the production of cellulases (Nisizawa et al., 1971). This could be due to the action of small amounts of $\beta$-glucosidase which splits sophorose to glucose which eventually accumulates to a level to cause 'Catabolite repression'.

In T. reesei coordinate induction of endo- and exo-glucanases occurs when sophorose is an inducer, while $\beta$-glucosidase production is constitutive. This was clearly demonstrated by Tanaka et al. (1979), as cellulase induction by sophorose needed some lag while no lag was observed for $\beta$-glucosidase. Similarly Nisizawa et al. (1971) found cellulase, xylanase and $\beta$-glucosidase activities in presence of sophorose, but only xylanase and $\beta$-glucosidase activities were observed when sophorose was not supplemented in the medium.

Experiments by Gong et al. (1978) showed that, antibodies specific for celllobiohydrolase (=exo-glucanase) failed to cross react with either purified cellobiase ($\beta$-glucosidase), purified endo-glucanase or crude endo-glucanase. These results indicate that endo-glucanase and
exo-glucanase have different physical structures and the three enzymes are transcribed and translated from three different genes. Navalainena and Palva (1978) reported a mutation in *T. reesei* that led to the loss of exo-glucanase, endo-glucanase, xylanase and mannase synthesis, however the activity of β-glucosidase was not affected. These results suggest that while the two enzymes of cellulase complex are regulated by a common regulatory circuit, β-glucosidase is regulated independently.

The recent isolation of constitutive mutants able to synthesize all cellulase components on a variety of non-inducing substrates indicates that cellulases are synthesized by repressor-inducer mechanism (Mishra et al., 1982). The lack of an active repressor or deletion of repressor site on DNA might have led to the constitutive character of all cellulase enzymes.

**Hemicellulases:**

Only limited information is available on the regulation of hemicellulases. Many of the available reports indicate that the regulation of xylanase synthesis is by inductive-repressive mechanism as occurs with many other polysaccharidases. (Daniel, 1982; Mullen and Bateman, 1972; Prabakaran, 1984; Srivastava, 1983, Strobel, 1963, Podile, 1986).
Coordinated induction of xylanase was reported for Cryptococcus albidus (Bailey et al., 1980). P. marginata (Srivastava, 1983). P. islandicum (Prabakaran and Dube, 1985a). In case of Bacillus coagulans endo-xylanase is inducible while $\beta$-xylosidase is constitutive (Estben et al., 1983).

Most of the arabinogalactan-degrading enzymes appear to be induced by arabinogalactan (Kaji and Yoshira, 1970; Mullen and Bateman, 1971) or by arabinose or structural derivatives (Cooper and Wood, 1973). L-arabinose is more effective inducer than D-galactose for galactanase or $\beta$-galactosidase in case in V. albo-atrum and F. oxysporum f. sp. lycopersici (Cooper and Wood, 1973). A degree of induction of arabanase synthesis by galactose also occurred. This is similar to the cross-induction of $\beta$-galactosidase of Neurospora crassa with arabinose than with galactose as inducer (Comp and Lester, 1971). In presence of arabinogalactan Penicillium islandicum produces only arabanase (Prabakaran, 1984), however Srivastava (1983) reported both arabanase and galactanase production by Pseudomonas marginata with arabinogalactan as substrate. To date the report of constitutive production of arabinofuranosidase by Penicillium digitatum (Cole and Wood, 1970) appears to be exceptional.
REGULATION OF CELL WALL DEGRADING ENZYMES in planta (=in vivo)

The synthesis of regulation of enzymes that degrade cell wall constituents appear to be under control of the pathogens. The best control is exemplified in the association of certain mycorrhizal fungi, which establish a compatible relationship with their hosts. Mycorrhiza are known to produce pectic enzymes (Williamson and Hadley, 1970). It is apparent that production of these enzymes by mycorrhizal fungi and obligatory parasites must be under adequate control, lest the invaded tissues become macerated and the host cells killed (see Bateman and Basham, 1976).

Isolated cell walls are a host factor of obvious relevance to regulation of CWDE synthesis in planta. The process of induction of CWDE by isolated plant cell walls has been more extensively studied with phytopathogenic fungi than with bacteria. CWDE are induced in a sequential manner over several days that begins with pectic enzymes followed by arabanase, xylanase and cellulase (Bateman, 1971; Cooper and Wood, 1975 English et al., 1971; Mullen and Bateman, 1972; Goodenough and Maw, 1976; Jones et al., 1972). The similar temporal sequence also seems to occur in infected tissue, as pectic enzymes are always the first enzymes to appear and cellulases the last. This sequence has been reported in plants infected by several organisms, including wilt fungi V. albo-atrum and F. oxysporum.

The sequence of CWDE production is presumably related to the accessibility of cell wall polymers and the sequential release of enzyme reaction products. It has been shown for *C. lindemuthianum* and *T. viride* as cell wall polymers are not amenable to the action of hemicellulases and cellulases; until the polygalacturonide of the cell walls has been degraded by endo-pectic enzymes (Karr and Albersheim, 1970; Keegstra et al., 1973). However contrasting results were obtained by Cooper et al. (1975) for *V. albo-atrum*, in which case individual polysaccharidase was able to degrade tomato cell walls without previous treatment of pectic enzymes; similarly an isozyme of AF of *Sclerotinia fructigena* could degrade apple cell walls independently (Laborda et al., 1974) and purified xylanase of *T. pseudokoningi* solubulizes xylose oligomers from cell walls of bean and corn (Bateman, 1976). Thus, fungi are able to recognize specific polymers in the cell wall and degrade it.

No significant differences in the pattern of enzyme secretion by fungi pathogens has been observed when cell
walls of susceptible or resistant plant varieties are used as a carbon source in liquid medium (Jones et al., 1972). However, inspite of the basic similarities in cell wall composition of monosaccharides (Anderson, 1978), cell walls derived from different host plants induce different enzyme synthesis in several fungi (Cooper et al., 1981). It is well established now, that pectic enzyme induction occurs prior to other enzymes on cell walls. Since galacturonan is covalently incorporated into the insoluble structure, direct uptake of this polymer is clearly precluded. So like most polymers here also the slight degradation by low levels of basal enzyme, releases products that induce the enzyme synthesis. It is also possible that soluble compounds present in the cell walls induce pectic enzymes. A low molecular weight water soluble fraction rich in uronic acid, from tomato cell walls, and water-soluble, ethanol-soluble components from elm shoots are effective inducers of polygalacturonases in F. oxysporum lycopersici and C. ulmi respectively (Biehn and Dimond, 1971; Jones et al., 1972).

Wilt fungi being in contact with cell walls in the metabolically inert xylem vessels, provides an unique system to study regulation of enzyme synthesis by host cell walls in situ (Cooper and Wood, 1980). In the transpiration stream of the host, localization of enzyme activity and inducers may occur with in extracellular
material which attaches hyphae to xylem vessels. Alternatively, hyphae may form intimate contact with the cell wall (Cooper and Wood, 1980). Basal enzyme levels may release sufficient inducer to initiate enzyme synthesis which may become autocatalytic. Exo-acting enzymes appear to play greater role in releasing inducers, since, some polysaccharides such as arabanases are invariably exo-acting and some pectic enzyme complexes act co-ordinately (both random and terminal), such as PG complexes of S. rolfsii and V. albo-atrum, PAL of E. chrysanthemi (Bateman, 1972; Garibaldi and Bateman, 1971; Mussel and Strouse, 1972). PGs of V. albo-atrum and C. lindemuthianum also act in dual fashion by first acting as exo-enzymes then as endo-enzymes (Bateman, 1972; Cooper et al., 1976; English et al., 1972). Such combined action is of obvious advantage to pathogens to cause extensive wall degradation and simultaneous release of monomers for nutrition and further enzyme induction.

Cell wall proteins capable of inhibiting the activity of endo-PGs secreted by plant pathogens have been reported from several dicotyledons (Albersheim and Anderson, 1971; Fisher et al., 1973). These inhibitors act on endo-PGs only and not against other CWDE (Albersheim and Anderson, 1971). This suggests that involvement of endo-PGs in the degradation of cell walls is a general phenomenon against which the plants guards by possessing proteins capable of blocking the action of these enzymes essential for
Plant cell walls also have a high binding affinity for certain proteins including polygalacturonide degrading enzymes from plants and fungi (Bateman, 1963; Byrde and Archer, 1976; Jansen et al., 1960; Skare et al., 1975). Such binding is probably ionic and these enzymes can be desorbed by high concentration of salts such as NaCl (Cooper et al., 1981). An alternate mechanism of restriction of activity of CWDE by cell walls per se may depend on variations between the porosity of walls and molecular size of enzymes.

Catabolite Repression: (C.R.)

Availability of readily metabolizable sugars such as glucose repress the synthesis of many catabolic enzymes. It is well known that operons of E. coli are positively and negatively controlled by glucose, which is known as 'glucose effect' or 'catabolite repression'. In E. coli cyclic adenosine monophosphate (c-AMP) serves as secondary messenger to signal whether glucose is available as cell fuel or not. E. coli also contain enzymes adenylate cyclase to make c-AMP from ATP and an enzyme phosphodiesterase which can hydrolyze c-AMP. In presence of glucose the enzyme phosphodiesterase hydrolyses c-AMP to inactive form. In absence of glucose, c-AMP binds to catabolite activator protein (CAP) and allows the entry site to bind RNA polymerase. Under these conditions RNA polymerase moves from
its entry site through the operator and begins to transcribe m-RNA.

The underlying principle of 'CR' is that, the inducers supplied at higher rates start accumulating and thus causing the inhibition of enzyme synthesis. In other words when the rate of catabolism exceeds that of anabolism the products of catabolism repress further enzyme synthesis, thereby by minimizing the energy consuming process.

Repression of CWDE synthesis by addition of glucose was shown for several organisms (Biehn and Dimond, 1971; Chatterjee et al., 1981; Cooper and Wood, 1975; Horton and Keen, 1966; Keen and Horton, 1966; Mullen and Bateman, 1971; Mussel and Green, 1970; Patil and Dimond, 1968; Spalding et al., 1973; Zucker and Hankin, 1971). Catabolite repression of pectolytic and cellulolytic enzyme synthesis by sugars is a common phenomenon and there is irrefutable evidence that this factor is important in pathogenesis. Horsfall and Dimond (1957) went to the extent of classifying plant diseases into "low sugar diseases" and "high sugar diseases". Usually the high sugar diseases involve obligate parasites while the low sugar diseases involve facultative parasites.

Horton and Keen, (1966) and Keen and Horton, (1966), examined the repression of cellulase and polygalacturonase
synthesis in *Pyranocheata terrestis* by glucose. Cellulase synthesis was repressed at above optimal concentrations of cellulose. This could be due to the excess rates of glucose liberated than the glucose utilized by the fungus. Cellulase synthesis in presence of cellulose was repressed to the basal level by glucose concentrations above $5 \times 10^{-4}$ M or above. However the rate of endo-polygalacturonase synthesis on pectin was double when $5 \times 10^{-4}$ or $5 \times 10^{-3}$ M glucose was added. Nevertheless it was repressed to approximately one tenth of the original rate by addition of $5 \times 10^{-2}$ M glucose. In infected roots, when sugar concentration was decreased to one-fourth by clipping the cotyledons, synthesis of both the enzymes increased and symptom development was also more rapid. When sugar concentration was increased by 25 or 75% by spraying glucose or meleic hydrazide respectively, synthesis of enzymes and symptoms development were reduced. Patil and Dimond (1968) suggested that the apparent inability of a pathogen to produce on enzyme could be due to catabolite repression, based on the effect of glucose on endo-PG activity. Glucose, when introduced into vascular bundles of *Fusarium* infected tomato plants, reduced the wilt symptoms by repressing PG production. Goodenough and Maw (1976) found that the major sugars in tomato roots were glucose (1.2%), fructose (1%) and inositol (0.7%). When these sugars were added at these levels to cultures of the root pathogen *P. terrestris*,
growing on cell walls, synthesis of CWDE was repressed upto 4 days.

Cooper and Wood, (1973) working with *V. albo-atrum* and *F. oxysporum lycopersici* reported synthesis of endo-PG and endo-PL which were shown to be increased almost linearly when supplied with galacturonic acid from '0' to about 10 mg per 100 ml/h but decreased rapidly at higher values, 90% inhibition was observed at 20 mg per 100 ml/h, at twice this rate repression of synthesis was almost complete. Similarly repression was observed for arabanase and /3-galactosidase but it was not complete. The results of pectic enzymes are interesting because of the evidence that implicates them in the development of the wilt syndrome. *Prima facie*, conditions in vascular elements would seem suitable for synthesis of these enzymes. The effect of sugars not specific in that repression is not confined to the end products of the action of a particular enzyme as suggested for other systems (Mc Fall and Mandelstam, 1963). The products of degradation of substrates of a range of enzymes, at low conc. repress synthesis of each enzyme in range (Cooper and Wood, 1975). However, the extent of repression depends on the enzyme and repressor. 'CR' of hemicellulases was reported in very few occasions. Srivastava (1983) reported repression of xylanase, arabanase and galactanase of *Pseudomonas marginata*. Prabakaran (1984) reported the repression of xylanase and arabanase
by glucose and several other sugars.

**Effect of c-AMP on catabolite repression**:

The release from CR by addition of c-AMP is reported for pectate lyase of *E. carotovora* (Huber et al., 1978; Tsuyumu, 1973). Chatterjee et al. (1981) reported PAL stimulation in presence of c-AMP + SDG (saturated digalacturonide) for *E. chryanathemi* and *E. caratovora pv. caratovora*. Prabakaran and Dube (1985b) reported release of CR of cellulase of *P. islandicum* by addition of c-AMP to the culture medium. Similar results were observed for xylanase and arabanase of *P. islandicum* (Prabakaran, 1984). Mandels and Reese (1975) noted that CR of cellulases of *T. reesei* can not be overcome by external addition of c-AMP.

Besides the possible effects of catabolite repression on enzyme synthesis by the pathogen, the pH of the environment in which the pathogen grows, and the influence of specific and non-specific inhibitors have marked influence on the stability of the enzyme. A typical example of the great influence of pH on enzyme activity is the activity of hydrolytic pectic enzymes at acidic pH and that of lyases at alkaline pH. The pectic enzymes present in the culture filtrates of *Rhizoctonia solani* differ with age and pH. Young cultures with an acidic pH primarily contain PG while older cultures having a shift of the pH to alkaline side
contain only PAL (Bateman, 1967). Many pathogens that produce pectic lyases do so best in an alkaline environment. Infection of tissues by a number of Fusaria, for example, results in alkaline pH of the infected tissues and pectic lyases are known to be the predominant pectic enzymes associated with these diseases (Bateman, 1966; Hancock, 1968; Mullen and Bateman, 1971). The dominant pectic enzymes found in tissues infected by S. rolfsii and S. sclerotiorum which create acidic environment by production of organic acids, are the polygalacturonases (PGs) which are active in acidic conditions (Bateman and Beer, 1965; Hancock, 1966).

Intermediary metabolites should also be considered as they can effect 'CR'. Metal co-factors seem to play greater role in regulating enzyme activity. Most of the regulatory enzymes are composed of more than one subunit, and it is postulated that intermediary metabolites exert their influence by reacting at the site on the enzyme different from the substrate and in doing so induce or stabilize the conformational change in the enzyme. In the case of inhibitor the enzyme shows less affinity towards the substrate while with stimulator it shows the opposite effect.

Regulation of production of cell wall degrading enzymes and their effectiveness in infected tissues is,
thus, directed by balance of forces at play in the environment created by the host-pathogen interaction. In compatible interactions the production of enzymes by pathogens that degrade plant cell walls appears to be the rule. (Bateman and Miller, 1966; Albersheim et al., 1969).

**Toxins:**

The toxin theory was proposed by Hutchinson (1913), when he was working with Rangapur tobacco wilt caused by *Bacterium solanacearum* (i.e. *Pseudomonas solanacearum*). In tobacco plants wilting of apical leaves occurred when only base of the stem was infected.

Several wilt organisms are reported to produce toxins in culture media as well as in planta (Asamov et al., 1975; Charudattan, 1970; Gour and Dube, 1985; Kalyanasundaram and Venkatram, 1956; Keen and Long, 1971; Keen et al., 1972; Lakshminarayanan and Subramanian, 1955; Nachmias et al., 1982). The media which are nearest to the conditions of in vivo consists of autoclaved products of the natural host (Rudolph, 1976).

Wilt toxins are classified into two groups depending upon their molecular weight. Toxins which have molecular weight below 1000 d are considered as low molecular weight toxins eg: Fusaric acid, lycomarasmin, fusicocin etc., high molecular weight toxins include glycopeptides produced by
P. solanacearum and PLP (protein lipopolysaccharide) of Verticillium spp. Many workers have excluded high molecular weight toxins because they appear to act in a physical rather than chemical manner (Van Alfen, 1982; Durbin, 1982). However, Strobel and Co-workers (Kai and Strobel, 1968) are of the opinion that several pathovars of Corynebacterium michiganense produce wilt-inducing macromolecules that affect plasmalemma function rather than mechanically occluding vascular elements.

F. oxysporum lycopersici produces lycomarasmin in substantial yields (300 mg/litre$^{-1}$) in static cultures after 40 days, tomato plants treated with the toxin developed symptoms of infection within 7 days, but the concentration of the toxin at this period is culture filtrate was 10 mg/litre$^{-1}$ only. But lycomarasmin has never been detected in vivo, it's involvement in symptom induction is at best only tenuous.

Fusaric acid (5-M-butyl picolinic acid) is produced as a cultural metabolite by several formae speciales of Fusarium oxysporum. It was the first vivo toxin reported in plant pathology. Lakshminarayan and Subramanian (1955) and Kalyanasundaram and Venkatram (1956) detected 17 μg/gm$^{-1}$ of fusaric acid per gm$^{-1}$ fresh weight of cotton tissues 2-3 weeks after inoculation with F. oxysporum vasinfectum. F. oxysporum lycopersici produces substantial quantities
of fusaric acid, 200 mg litre$^{-1}$ after 200 days in still culture or 6 days in shake culture.

*Fusicoccum amygdali* produces a wilt toxin *fusicoccin* which causes a rapid wilting. Dessication and death of the shoot, follows in the distal branches uncolonized by the pathogen. Under conditions of moisture tension, tomato shoots, treated with 2 µg *fusicoccin* g$^{-1}$ fresh-weight, showed an increased rate of transpiration over water uptake with a concomitant loss of turgor. No effect on vascular flow was observed, the transpiring cells were not killed before losing water.

Both the wilt inducing species of *Verticillium* viz. *V. dahliae* and *V. albo-atrum*, produce PLP complexes in cultures. Porter and Green (1952), were the first to suggest that culture filtrates contained a large toxic molecule, but no characterisation was attempted. Zel'tser and Malysheva (1966) and Malysheva and Zel'tser (1968) described a wilt-inducing PLP complex from cultures of *V. dahliae*, that were active on cotton. Keen and Long (1972) and Keen et al., (1972) also reported the same. The authors claimed that excised cotton leaves supplied with 5 µg ml$^{-1}$ of the PLP from DEAE cellulose, showed typical wilting and necrosis. They also claimed that purified toxin induced severe symptoms in susceptible plants, intermediate symptoms in moderately-susceptible cultivars,
and mild symptoms in resistant cultivars.

Asamov et al. (1975) claimed that a polypeptide and an oligosaccharide of *V. dahliae* changed the permeability of artificial phospholipid membrane to Na\(^+\) and K\(^+\) ions. Nachmias et al. (1982) reported the isolation of a host-specific PLP complex from cultures of potato isolate of *V. dahliae* which was more toxic to susceptible cvs. of potato than to resistant cvs.

Recently Gour and Dube (1985) reported host-specific phytotoxic metabolites from *V. dahliae* cultures, toxic to the susceptible cv. of cotton, but not to three resistant cvs. Cuttings from 8 week old cotton plants of the susceptible cv. developed typical wilt symptoms 8 h after being treated with the toxin preparation. Loss of electrolytes from leaf and root tissues was significantly greater than the water-treated control. Root tissues of susceptible cv. also showed a significant loss of /5-cyanin pigments within 15 minutes of exposure to the toxin preparation. Significant losses of K\(^+\) and Na\(^+\) occurred from toxin treated leaf tissues but permeability to Ca\(^{+2}\) was not affected.

The visual symptoms generally exhibited by toxin-treated plants include, chlorosis, necrosis, wilting etc. (Strobel, 1974) but these expressions tell us very little about a toxin's mode of action. Little is known about the mechanism or sites of action of any toxin, particularly
those involved in wilt diseases. Some of the mechanisms proposed for action of wilt-phytotoxins are: disruption of cell membranes (Linskens, 1955) and reduction of flow through the stem by either vascular plugging or increase in viscosity of the xylem sap (Van Alfen and Turner, 1975, Husain and Kelman, 1958). Gowda and Rai (1980) claimed that wilting caused by P. solanacearum was not due to the plugging of the vessels as the toxin diffused in the plant system faster than the pathogen itself and caused wilting. Recent work of Gour and Dube (1965) with ouabian, an ion transport inhibitor, suggests that, Vd toxin-sensitive K$^+$ and Na$^+$ ion transport systems are present in plasmamembranes of susceptible cv. of cotton plants but not in the resistant cv. These sites are sensitive to ouabian treatments.

The primary mechanism of wilting induced by fusaric acid (FA) was explained as damage of plasma membranes as evidenced by increased secretion of ions (Linskens, 1955). Researchers at Madras. Gnanam, 1956; Sadasivan and Kalyanasundaram, 1958; and Sadasivan and Saraswathi-Devi, 1957) demonstrated ionic imbalance in Fusarium-infected cotton plants. Similar loss of electrolytes from Verticillium-infected plant tissues was reported by Dube, (1971).
It has been shown in several instances that FA production by *F. oxysporum lycopersici* in vitro varied considerably with the composition of the medium, and virulent and avirulent isolates reacted differently to the changes in the composition of the medium (Sanwal, 1956). The failure to demonstrate the *in vitro* toxin, in plant, such as lycomarasmin could be due to our inability to simulate *in vivo* conditions *in vitro*. It may also be due to several other factors such as liability of toxin, binding of toxin to sites of action from which it cannot be liberated without disruption.