Literature Review

Plants respond to infection by producing physical and chemical barriers which function as wall reinforcements, antibiotics or lytic enzymes (Nemestothy and Guest, 1990), It has been established in detailed comparative study of resistant and susceptible host responses that the differences between them are quantitative rather than qualitative. Although susceptible plants possess the machinery necessary for resistance it is not activated in sufficient magnitude or speed to restrict the infection (Kuc 1983). The success or failure of infection is also determined by dynamic competition and final outcome is determined by the sum of favourable and unfavourable conditions for both the pathogen and host cell. Antigenic similarity between host and pathogen as a prerequisite for compatable host pathogen interaction has also been established.

An attempt has been made in the following pages to review available literature on biochemical changes in plants following infection and serological relationship between host and pathogen.

Biochemical changes in plants following infection

Biochemical changes in plants following infection or attempted infection by pathogens has been a subject of research by a number of workers (Ji and Kuc, 1996; Wibber et al., 1996; Kristensen et al, 1997). Accumulation of phytoalexin as defence response has been demonstrated in more than hundred plant species till now. Several reviews have appeared on the subject (Ward 1986; Lamb et at. 1989 ; Keen, 1990 ; Chakraborty et al. 1995, Purkayastha, 1995; Oku and Shiraishi, Kuc,1995).

Metraux and Boller (1986) reported that in the first leaves of young cucumber plants infected with fungal, bacterial or viral pathogens, the activity of chitinase (E.C.3.2.1.14) increased up to 600 fold in the infected areas of the leaf. In uninfected areas of infected leaves, chitinase was induced about 10 times less strongly. Chitinase was induced up to 100 fold in the uninfected second leaves of the plants. The increase of chitinase activity in the second leaves correlated well with an increase in resistance against a challenge infection by *Colletotrichum lagenarium.*Chitinase could also be induced about 10 fold by treatment with 10nl ml^{-1} ethylene for 27h. These plants showed enhanced resistance to infection by C. *lagenarium.* Treatment of the first leaves

of cucumber plants with necrotizing salt solutions caused a 20 to 50 fold increase of chitinase activity in the first leaves, a three to seven fold increase in the untreated second leaves and a slightly enhanced resistance against infection by C. *lagenarium.*

The expression of resistance and susceptibility to inoculation with zoospores of *Phytophthora megasperma* f. sp. *glycinea* race I was determined in roots, hypocotyls, and cotyledons of etiolated and green seedlings and in leaves of soybean cvs. Harosoy (rps_1) and Harosoy 63 (Rpsl). Gene-specific resistance was demonstrated in all organs tested, except for cotyledons of etiolated seedlings. In each case higher concentrations of the glyceollins accumulated in resistant than in susceptible reactions; the difference being greatest in hypocotyls and smallest in roots. The relative proportions of glyceollin I, II and III varied with the organ, exposure of the seedlings to light, the interaction type and the incubation period. Glyceollin I was relatively the most abundant isomer in roots and to a lesser extent in hypocotyls. Glyceollin III was relatively the most abundant isomer in leaves. Major differences in accumulation rates were observed in time-course experiments and, after reaching a maximum, all three isomers decreased in leaves and glyceollin I decreased in hypocotyls. Bhattacharyya and Ward (1986) suggested that concentrations and hence isomeric proportions were finally controlled by rates of biosynthesis and metabolism.

The authors further determined phenylalanine -ammonia lyase (PAL) activity in soybean hypocotyls at 25 or 33°C following infection with *Phytophthora megasperma* f. sp. *glycinea* or treatment with the abiotic elicitor, $AgNO_3$. PAL activity was less at 33°C than at 25°C in each of six cultivars examined but was lowest in two cultivars previously shown to develop susecptibility at 33°C Glyceollin accumulation was determined in response to $Ag\ NO_3$ treatment and was higher at 33°C than at 25°C in four cultivars tested. The increase was marginal with two temperature sensitive cultivars but more than 50% in two cultivars that remained resistant at 33°C. There were significant differences among 18 races of the pathogen for growth rates *in vitro.* The effect of temperature (25 or 33°C) on growth, sensitivity to glyceollin I and the interaction of temperature and glyceollin I sensitivity. Growth of some races (eg-race 2) was strongly inhibited at 33°C, that of others was similar at both temperatures,

and that of one (race 19) was faster at 33°C than at 25°C. Minimal restriction of growth at 33°C and relative tolerance to glyceollin I in race 4 combined with a major suppression of PAL activity and little increase in glyceollin accumulation at 33°C in Cv. Altona was consistent with temperature induced susceptibility in this race-cultivar interaction. The possibility that combinations of physiological variables in host and pathogen, rather than or in addition to, putative recognition systems, may define reaction type has been proposed. (Bhattacharyya and Ward, 1987).

Bhattacharyya and Ward (1987a) also reported that in unwounded soybean hypocotyls, pulse labelled with [^{14}C] phenylalanine and inoculated with *Phytophthora megasperma* f. sp. *glycinea,* rates of [^{14}C] incorporation and glyceollin I accumulation were higher in resistant than in susceptible responses throughout the time course of the experiment. This distinction was maskd in hypocotyls that were wounded and inoculated. In such hypocotyls, high rates of [^{14}C]-incorporation developed that were similar for the first 11 h in resistant and susceptible responses, although much more glyceollin I accumulated in the former. High rates of [^{14}C]-incorporation also developed in uninoculated wounded hypocotyls but only small amounts of glyceollin I of high specific radioactivity were detected. Estimates of phenylalanine ammonialyase activity indicated that the metabolic flux through phenyl alanine was limited in wounded controls but potentially very high in resistant responses. Differences in rates of [^{14}C]-incorporation and in specific radioactivity of accumulated glyceollin I presumably indicate differences in the relative contributions of mobile internal pools and externally applied phenylalanine, in addition to rates of biosynthesis. Rapid decline in [^{14}C] glyceollin I was demonstrated in wounded controls in pulsechase experiments with phenylalamine as chase, but not in inoculated hypocotyls, due to continued [^{14}C]-incorporation during the chase period. Rapid metabolism was demonstrated in all interactions and in wounds when cinnamic acid was used as the chase, but there was no evidence that differences in glyceollin I accumulation were due to differential rates of metabolism. Additional evidence for metabolic activity was provided by pulse feeding with [^{14}C] glyceollin I.

A glucan elicitor from the cell walls of the fungus *Phytophthora*

megasperma f.sp. *glycinea* also caused increases in the activities of the phytoalexin biosynthetic enzymes, phenylalanine ammonia - lyase and chalcone synthase, and induced the production of the phytoalexin, glyceollin, in soybean *(Glycine max)* cell suspension cultures when tested in culture medium containing 1.2 mmol / liter Ca^{2+} (Stab and Ebel, 1987). Removal of extracellular Ca^{2+} by treatment with ethylene glycol bis (β-aminoethyl ether)-N,N'-tetraacetic acid followed by washing the cells with Ca^{2+} free culture medium abolished the elicitor - mediated phytoalexin response. This suppression was largely reversed on readdition of Ca^{2+} , Elicitor - mediated enhancement of biosynthetic enzyme activities and accumulation of glyceollin was strongly inhibited by La^{3+} ; effective concentrations for 50% inhibition were (μmol / liter)/ 40 for phenylalanine ammonia - lyase, 100 for chalcone synthase, and 30 for glyceollin. Verapamil caused similar effects only at concentrations higher than 0.1 mmol / liter, whereas trifluoperazine and 8-(diethyl amino) - octyl - 3,4,5- trimethoxybenzoate did not affect enzyme induction by the elicitor in the concentration range tested. Uptake of $\mathcal{L}$-amino isobutyric acid into soybean cells, which was rapidly inhibited in the presence of the glucan elicitor, was not affected by La^{3+} nor was uptake inhibition by the elicitor relieved by La^{3+} . The Ca^{2+} ionophore, A 23187, enhanced phytoalexin biosynthetic enzyme activities and glyceollin accumulation in a close - dependent manner, with 50% stimulation (relative to the elicitor) Sodium azide was found to be most effective of the six metabolic inhibitors tested in reducing charcoal rot disease of soybean (cv. soymax) caused by *Macrophomina phoseolina* (Chakraborty and Purkayastha, 1987) Glyceollin production also increased significantly after induction of resistance by sodium azide treatment occurring at about 5 μmol / liter.

In another study Esnault et al (1987) determined the production of mRNAs for phenylalanine ammonia lyase and chalcone synthese in the first 5h following infection of intact etiolated soybean hypocotyls with zoospores of *Phytophthora megasperma* f.sp. *glycinea* mRNA was extracted from tissue excised from inoculated sites and mRNAS for the two enzymes detected by dot hybridization using corresponding cDNA probes. A major increase in mRNAs for both enzymes was detected by 3h following inoculation in an incompatible interaction but not in a compatible interaction. The results are consistent with the development of early differences in glyceollin biosynthesis in the two types of interaction.

Phenylalanine ammonia-lyase activity was reported to increase rapidly beginning 2h after inculation with *Phytophthora megasperma* (Drechs.) f. sp. *glycinea* (Hildeb). Kuan & Erwin race 1 in unwounded hypocotyls of soybean cv. Harosoy 63 (resistant) but did not change significantly in cv. Harosoy (susceptible). Small increases in phenyl alanine ammonia lyase activitiy also were caused by wounding. Activity increased more slowly in hypocotyls (cv. Harosoy 63) wounded just before inoculation than in intact inoculated hypocotyls, but most activity developed in hypocotyls wounded 12h before inoculation. There were comparable effects of wounding on symptom development. Trifoliate leaves of 14 day - old cv. Harosoy 63 plants are resistant, but trifoliate leaves of 12 day old cv. Harosoy 63 plants and 14 day old cv. Harosoy plants are susceptible to race 1. Increase in phenylalanine ammonia lyase activity following inoculation were demonstrated only in 14-day old Harosoy 63 plants but not until 24-36 h after the inoculation. Significant accumulation of glyceolin occurred by 24h. Susceptible trifotiate leaves of 12 day old cv. Harosoy 63 plants produced only low levels of glyceollin following either infection or treatment with the abiotic elicitor Ag NO_3, whereas trifotiate leaves of 14 day old cv. Harosoy plants produced high levels of glyccollin in response to $AgNO_3$. The authors concluded that trifoliate leaves of 12 day old, as opposed to 14 day old, cv. Harosoy 63 plants have not developed mechanisms that trigger responses to either infection or the abiotic elicitor or they are defi cient in metabolic processes that support glyceollin biosynthesis or other defence related responses (Bhattacharyya and Ward, 1988).

A pathogenesis related (PR) protein was found in both the infected and the uninfected leaves of cucumber plants inoculated on the first true leaf with a fungal, a bacterial or a viral pathogen. This host coded protein was detected up to five leaves above the infected leaf. The protein was purified from the intercellular fluid by ion- exchange chromatography and by high performance liquid chromatography on ion- exchange and phenyl sepharose columns. The purified PR-protein was shown to be a chitinase with a molecular mass of 28000 as determined by SDS polyacrylamide gel electrophoresis and by gel filtration (Metraux *et al*; 1988). Changes in the soluble protein pattern of leaves of eight lines of barley, carrying different resistance genes to mildew, were analysed by SDS polyacrylamide gel electrophoresis and isoelectric focusing (Bryngelsson

et al; 1988). Apparently new host proteins were induced by *Erysiphe graminis* f.sp. *hordei* in the incompatible reaction which were not present in the immune or susceptible response. These proteins are of low molecular weight, 13,500-27,000 d, and have either very low or very high isoelectric points. Thus, they resemble the pathogenesis related proteins found in many dicotyledonous species. In a further study, one of the pathogenesis related proteins, HV-1, in barley was isolated and characterized. *Hordeum vulgare* cv. Alva was challenged with an incompatible race of mildew *(Erysiphe graminis* f. sp. *hordei)* and the HV-1 protein was isolated from necrotized tissue by a combination of ammonium sulphate precipitation, anion exchange chromotography, hydrophobic interaction chromatography and chromatofocusing. The protein has a molecular weight of approximately 19KD and an isoelectric point of 3.4. It is not a glycoprotein as judged from its lack of reaction with concanavalin A and peanut agglutinin. The HV-1 protein was partially sequenced and compared to known protein sequences. The sequence of 28 amino acids had a 48% homology with the N-terminus of osmotin and NP24, proteins which are synthesized in response to salinity stress in tobacco and tomato, respectively. The same level of homology was also present in a maize trypsin / $\mathcal{L}$- amylase inhibitor and in thaumatin, an intensely sweet tasting protein from the West African shrub *Thaumatococcus daniellii* Benth. Even higher homology (58%) was discovered with the terminus of the PR-5 protein of tobacco (Bryngelsson and Green, 1989).

The effects of foliar application of growth substances and mineral nutrition of the host on the development of charcoal rot disease of soybean caused by *Macrophomina phaseolina* was tested by Chakraborty et al. (1989). Among the eight growth substances examined, gibberellic acid was most successful in reducing the disease serverity, followed by 3-indoleacetic acid and 2,3,5-triiodobenzoic acid. Low concentrations of these compounds stimulated while high concentrations inhibited the mycelial growth of *M. phaseolina invitro.* Substrate supplementation with different doses of N, P, K and Ca had varying effects on disease development. Disease increased considerably by both excess and deficient N and also by deficient Ca, while excess Ca conferred partial resistance. Glyceollin contents of host roots before and after excess Ca and gibberellic acid (10mg / L) treatments were estimated. Both compounds

significantly increased glyceollin production in infected roots. However, gibberellic acid induced glyceollin synthesis even in uninoculated roots. Changes in the host reaction towards increased resistance was correlated with increased phytoalexin production.

The possibility that susceptibility of immature leaves of soybean was due to deficiencies in metabolic precursors required for the expression of resistance and glyceollin synthesis was tested by supplying a range of compounds to the leaves prior to inoculation (Ward, 1989). These included sugars, glyceollin precursors and growth factors. Only glucose, galactose and sucrose were clearly effective and they promoted resistance to *Phytophthora megasperma* f. sp. *glycinea* race 1 in both cv. Harosoy 63 and in the normally susecptible cv. Harosoy. In both cvs supplied with glucose, resistance was associated with the accumulation of significant levels of glyceollin. More immediate precursors of glyceollin were ineffective and the possibility that glucose functions as a source of energy required for the development of resistant responses or for leaf maturation rather than as a source of glyceollin precursors has been discussed.

The differential regulation of the activities and amount of mRNAs for two enzymes involved in isoflavonoid phytoalexin biosynthesis in soybean was studied by Habereder et al. (1989) during the early stages after inoculation of primary roots with zoospores from either race -1 (incompatible host resistant) and race -3 (compatible, host susecptibility) of *Phytophthora megaspermum* f.sp. *glycinea,* causal fungus of rot disease. In the compatible interaction cloned cDNAs were used to demonstrate that the amount of PAL and chalcone synthase mRNA increased rapidly at the time of penetration of fungal germ tube into epidermal cell layer (1-2h after inoculation) concomitant with the onset of phytoalexin accumulation highest levels were reached after 7 h in the compatible and incompatible interaction Saindrenan et al. (1989) reported that the treatment of detached cowpea leaves with phosphite, the active breakdown product in plant tissues of fosetyl AL, lead to the cessation of growth of *Phytophthora cryptogen* within 24 h of inoculation. Pretreatment of leaves with $\mathcal{L}$-amino oxyacetate (AOA) an inhibitor of phenylpropanoid pathway increases the size of lesion in phosphite treated leaves, and induce a complete susecptibility. By 24h. after inoculation, PAL activity is higher in phosphite treated leaves than in

untreated. The effect of AOA on PAL activity are parallel with the effect of the increase of the spread of infection. The effect of ethylene on the induction of PAL by cell wall elicitor of *Colletotrichum lindemuthianum* was investigated by Hughes and Dickenson (1989) in leaves of susceptible and resistant cultivar of *Phaseolus vulgaris.* Induction of PAL without elicitor was negligible.

Li *et.al.* (1989) reported that extract from Hamimelon plant immunized with *Colletotrichum lagenarium* could effectively inhibit the growth of *Phytophthora melonis.* Activities of three enzymes in phenyl propanoid metabolism i.e. phenyl alanine ammonia lyase (PAL), cinnamate - 4 hydroxylase (CA4H) and 4-coumarate : COA ligase (4CL) were stronger in immunized plants. The activity of peroxidase was also significantly higher in immunized plant. A new protein was identified from immunized plants by 10% polyacrylamide gel electrophoresis, which had an isoelectric point of p^H 5.0 and a molecular weight of about 15.5 KD. The protein did not directly inhibit the germination of P. *melonis* conidia. Studies on the cell suspension cultures of bean *(Phaseolus vulgaris)* cv. *Imuna* which accumulated isoflavonoid phytoalexin on exposure to elicitor from the phytopathogenic fungus *Colletotrichum lindemuthianum* was carried out by Ellis et al. (1989). Phytoalexin accumulation was preceded by rapid increase in the activities of phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS). The relative levels of transcript from individual member of the CHS multigene family differ significantly at 1.5 h compared to 22.5 h after elicitation. More strikingly, three PAL genes were expressed in cultivar Imuna in response to fungal elicitor, whereas two are expressed in elicitor treated cell cultures Canadian order.

The effect of the product of the PAL reaction, transcinnamic acid (CA), on the appearance of individual PAL transcript in suspension cultured bean cells was studied by Mavandad *et al* (1990). Concentration of CA in excess of 10^{-4} mole inhibited appearance of elicitor induced transcript encoding PAL1, PAL2 and PAL3 when added to the cell at the same time as fungal elicitor. Addition of CA 4 hours post elicitation caused a major reduction in levels of all three PAL transcript, but with different kinetics and subsequent rates of recovery. The inhibition of accumulation of PAL 1, PAL 2, or PAL 3 transcript measured 3 h. after exposure to elicitor, as a function of the time of addition of CA postelicitation reflected the different rates of appearance of the three PAL

transcripts in the presence of elicitor alone. The inhibitory effect of CA as seen on PAL transcript were not observed for the constitutively expressed transcript H1, or the elicitor inducible 1,3-β - D glucanase. Analysis of *in vitro* translated polypeptides showed that some elicitor induced mRNA activities were not down regulated by CA, and that a number of other mRNA activities were induced by CA, thus providing further evidence for specificity in the action of CA on bean cells. Treatment of elicited cells with L-$\mathcal{L}$aminoxy-β phenyl propionic acid, a potent specific inhibitor of PAL activity, resulted in maintenance of elevated PAL transcript level beyond 12 h post elicitation, this effect being greatest for PAL transcript 2 and 3. .

Nemestothy and Guest (1990) reported that No. 2326, a cultivar of tobacco resistant to race O of the blank shank pathogen *Phytophthora nicotianae* var. nicotianae responded to stem inoculation of tobacco by rapidly accumulating sesquiterpenoid phytoalexins and activating phenylalanine ammonia lyase activity at the infection front. In cv. Hicks, a near-isogenic susceptible cultivar, both responses were slower. Pretreatments of leaf discs with propylene oxide, which killed the cells, mevinolin, a specific inhibitor of sesquiterpenoid biosynthesis, or the non-specific amino-transferase inhibitor, aminoxyacetic acid (AOA), inhibited post infection phytoalexin accumulation in both cuttivars, and induced susecptibility in cv. NC 2326. Amino hydrazinophenyl propionic acid (AHPP), a specific inhibitor of phenylalanine ammonia lyase activity and aminoeth oxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis, do not affect the susecptibility of either cultivar.

Plants of the cv. Hicks are protected from infection by the systemic phosphonate plant protectant, fosetyl-Al. Sesquiterpenoid phytoalexins, lignin and ethylene accumulated, and phenylalanine ammonia lyase activity increased more rapidly in fosetyl - Al-treated Hicks stems than in untreated stems. Propylene oxide, mevinolin and AOA inhibited sesquiterpenoid phytoalexin accumulation and the effectiveness of fosetyl - Al in cv. Hicks. Fosetyl - Al did not enhance sesquiterpenoid phytoalexin biosynthesis in cv. NC 2326, and only marginally reduced pathogen growth in the initial stage of infection, before resistance was expressed. Mevinolin and AOA did not induce total susceptibility in fosetyl-Al treated NC 2326, indicating that factors other than sesquiterpenoid phytoalexins are also involved in the mode of action of fosetyl - Al in this cultivar.

Stem injection of tobacco cultivar Ky 14 with *Peronospora tabacina* or leaf inoculation with tobacco mosaic virus induced systemic resistance to both pathogens. The treatment also elicited a systemic increase in peroxidase activity which was positively correlated with induced resistance. Increases were evident in cytosol, intercellular fluid, and cell wall fractions. Upon challenge with *P. tabacina,* peroxidase activity further increased in the induced plants and remained higher after challenge as compared to the control plants. The isozyme patterns of peroxidase on isoelectric focusing gels showed an increase of two anionic peroxidases. Both peroxidases were positively correlated with induced resistance (*Ye et al.* 1990). They further reported that inoculation of three leaves of tobacco cv. Ky 14 with tobacco mosaic virus and incubation at 23°C for 3-12 days caused localized necrosis on the inoculated leaves where as plants held at 28°C after inoculation developed systemic mosaic symptoms. Pathogenesis - related (PR) proteins and activities of peroxidase, β-1.3 glucanase and chitinase systemically increased in the inoculated plants at 23°C. When TMV inoculated leaves were removed 12 days after inoculation at 23°C and plants were challenged with TMV or *Peronospora tabacina* and held at 23°C, induced resistance to *P. tabacina* and TMV was apparent. However, resistance to blue mould but not TMV was apparent when the procedure was repeated, except that plants were transferred to 28°C one day after challenge with TMV or P. *tabacina.* PR proteins and activities of peroxidase, β-1,3 glucanase and chitinase were further increased after challenge with P. *tabacina* or TMV in induced plants at both temperatures. Tn 86, a cultivar systemically susecptible to TMV, was protected against blue mould but not against TMV by stem inoculation with P. *tabacina* PR proteins were induced and the activities of peroxidase, β-1,3 glucanase and chitinase were greatly increased in the induced plants (Ye et al; 1990).

It has also been observed/pointed out that light / sucrose treatment of sunflower (*Helianthus annuus*) hypocotyl originated cyclic changes of PAL activity with alternative periods of increase and decrease of activity and maxima at 4 and 28 h (Jorrin et al 1990). Actinomycin D, corelycepin and cycloheximide inhibited both induction and decay of PAL activity. These result suggested that the turnover mechanism of PAL implicates *denovo* synthesis of enzyme, better than activation of inactive precursors, in response to the inducer agent (light

and sucrose) and subsequent synthesis of a putative proteinaccous PAL inactivating system responsible for PAL activity decay. Expression of PAL gene during tracheary element differentation was studied in mesoplyll cell suspension cultures of *Zinnia elegans.* Dose response curves of benzyl adenine (BA) and $\mathcal{L}$-naphthalene acetic acid (NAA) were obtained for the cultures in order to achieve the highest percentage. Of BA and NAA, 0.1 mg. l^{-1} and 0.06 mg. l^{-1} respectively, normally stimulated about 40% differentiation by 96 h of culture. The effect of same ratio but different amount of BA and NAA on tracheary element formation have been tested and the result indicated that the absolute amount of BA and NAA rather than the ratio of them were important for tracheary element formation in the *Zinnia* cultures. The cells when cultured in the presence of 0.001mg $.l^{-1}$ of BA and 0.06 mg. $.l^{-1}$ of NAA expanded and divided but did not differentiate. The level of PAL activity, synthesis of PAL protein and level of PAL mRNA peaked during 72 to 96 h when lignin was actively deposited. This indicated that the PAL gene was temporally and preferentially expressed in association with the lignification during tracheary element differentiation and thus it can be regarded as a molecular marker for the process. (Lin and Northcote, 1990).

In a study with carrot *(Daucus carota* L.cv. *kurodagosun)* suspension culture cells, PAL was slowly induced during anthocyanin synthesis which occurred in a medium lacking 2,4 dichlorophenoxy acetic acid and was also induced rapidly and transiently by transferring and diluting cells to fresh medium. Analyses of nucleotide sequence derived from PAL cDNAs revealed that the PAL mRNAs induced by transfer were transcribed from different carrot PAL genes than the PAL mRNAs induced during anthocyanin synthesis. Northern blotting, using probes derived from 3' non-coding region for PAL cDNAs confirmed that different PAL genes were induced during anthocyanin synthesis and after transfer. Induction of different PAL genes occur in response to difference in induction trigger (Ozeki *et al*, 1990). The timing of changes in protein synthesis pattern of elicitor treated [^{35}S] methionine labelled parsley cell *(Petroselinum crispum)* was analysed by two dimensional gel electrophoresis Five groups were distinguished from a large number of elicitor responsive as well as unresponsive proteins. Two groups were synthesized *denovo* either early or late after elicitor application; two other groups were

strongly reduced in their rates of synthesis either early or late after elicitor application; and one group was not appriciably affected at all. The elicitor induced changes altered the total protein composition considerably. A few selected, induced protein were functionally identified. These included two early induced enzymes, (PAL) and 4-coumarate : CoA ligase (4CL) and a late induced enzyme, a bergaptol - O methyl transferase (BHT) which is specifically involved in the biosynthesis of furanocoumarin phytoalexin. The biological significance of the observed differential timing of changes in protein synthesis rates was discussed by Bollmann et al (1990).

Alfalfa (*Medicago sativa L.)* cell suspension cultures accumulated high concentration of the pterocarpon phytoalexin medicarpin, reaching a maximum within 24 hours after exposure to an elicitor preparation from cell walls of the phytopathogenic fungus *Colletotrichum lindemuthianum* (Dalhim et al. 1990). This was preceded by increase in the extractable activities of the isoflavonoid biosynthesis enzyme L-phenyl alanine ammonia lyase, cinnamic acid 4-hydroxylase, 4-coumarate Co-A-ligase, chalcone synthase, chalcone isomerase and isoflavone O-methyl transferase. Pectic polysaccharides were weak elicitors of phenylalanine ammonia lyase activity but did not induce medicarpin accumulation, whereas reduced glutathione was totally inactive as an elicitor in this system. The fungal cell wall extract was a weak elicitor of the lignin biosynthetic enzymes caffeic acid O-methyl transferase and coniferyl alcohol dehydrogenase, but did not induce appreciable increase in the activity of the hydrolytic enzymes chitinase and 1.3 β-D-glucanase. Accumulation of polyphenoloxidase, peroxidase and phenylalanine ammonia lyase from cucumber *Cummis sativus* L. leaves were studied during the period of 22 days after inoculation with cucumber powdery mildew. Results indicated that early rapid increase of polyphenloxidase, peroxidase and phenylalanine ammonia lyase activities was of great significance in the disease resistance.

The 1- Amino - 2 phenylethyl phosphonic acid (phep) stimulated growth in *Allium cepa* L grown *in vitro* for 30 days, and increased activity of phenylalanine ammonia lyase both per mg fresh wt. and mg protein. After 60 day phep reduced PAL activity (Knypl and Janas, 1990). It was also shown by Janas and Knypl (1990) that 1-amino 2-phenyl ethyl phosphonic acid (phep) enhanced phenyl alanine ammonia lyase activity (PAL) upto 7-6 fold times,

increased the level of phenylalanine upto 8 times and reduced amaranthin content in *Amaranthus caudatus* L., the effect being dependent on age of seeding, illumination, concentration and optical enantiomer of phep. D(+)-phep was a stimulator of PAL *in vivo* whereas L (-) - phep at 0.01 and 0.1 mM reduced PAL activity by about 20%, and stimulated it at 1mM. The inhibitory effect of phep on amaranthin accumulation was strengthened by L (-) phenylalanine which at 0.01 and 0.1mM stimulated amaranthin accumulation, phep reduced PAL activity and potentiated the stimulatory effect of D(+) phep on PAL. PAL activity in the seedlings oscillated at 12 h intervals; the oscillation pattern was not modified by phep.

Akhtar and Garraway (1990) reported that the increase in peroxidase activity and electrolyte leakage induced in maize (*Zea mays* L.) leaves by sodium bisulfite were causally related to the sodium bisulfite induced increases in sporulation of the pathogen *Bipolaris maydis* race T on infected maize leaves. Pretreatment of detached leaves of maize inbred W64A with sodium bisulfite (500 μg/ml) for 24 h in the dark at 28^{0}C increased peroxidase activity in the Tms cytoplasm (susceptible) isoline compared with the N cytoplasm (resistant) isoline. No such differences in peroxidase activity between the two isolines were observed when detached leaves were pretreated with double distilled water. The sodium bisufite induced increase in peroxidase activity persisted even when leaves pretreated with sodium bisulfite were inoculated with *B. maydis* race T and subsequently incubated for 48 h in the dark at 28^{0}C. Similarly, pretreatment with sodium bisulfite caused a greater increase in electrolyte leakage as well as in sporulation on the leaves of the susceptible than on those of the resistant isoline when compared with leaves not treated with sodium bisulfite. Sodium bisulfite showed no effect on sporulation *in vitro.* Leachates from the susceptible isoline pretreated with sodium bisulfite also caused greater increase in sporulation than those from the resistant isoline pretreated with sodium bisulfite.

In another study, phenylalanine ammonia lyase (PAL) and peroxidase activities were shown to increase slightly during prehaustorial stages of development of the leaf rust fungus *(Puccinia recondita* f.sp. *tritici)* in each combination of rust strain and wheat line used. Activity of both enzymes greatly increased during the formation of the first haustoria by avirulent and virulent

strains in an Lr 28 bearing line but not in an Lr 20-bearing line. PAL increased markedly in activity at the time when an avirulent strain was known to cause hypersensitivity in the Lr 28 bearing line and when another avirulent strain was known to cause cellular changes that preceded the expression of resistance in the Lr 20 bearing line. PAL increased less markedly and not at all at corresponding times when virulent strains developed in the Lr 28 and Lr 20 lines respectively. Decrease in the peroxidase activity occurred after the increase in PAL activity and were greater during resistance expression than in leaves infected with virulent strain. Cytochemical tests revealed enhancement of peroxidase activity in epidermal and guard cells in response to avirulent and virulent strains only. The implication of the changes are that aromatic and phenylpropanoid synthesis and peroxidation may be enhanced in wheat by infection and particularly during resistance expression. The products of these processes may play roles in both Lr 28 and Lr 20-based expression of resistance (Southerton and Deverall, 1990).

Spraying the lower surface of the first true leaf (leaf 1) of cucumber plants with 50 mM K_2HPO_4 induced systemic resistance to anthracnose caused by *Colletotrichum lagenarium,* 2 to 7 days later (Irving and Kuc, 1990). Within 16 h of application, the activities of peroxidase and chitinase increased in the induced leaf, and they continued to increase over the next 7 days reaching levels at least ten fold higher than in leaf 1 of plants treated with water. During this period, the activities of both enzymes were usually two-fold or higher in leaf 2 of plants induced with K_2HPO_4 than in plants treated with water. If the induced plants were not further treated or mock challenged with water on leaf 2, the enzyme levels increased slightly and levelled out at between two or four fold higher than in the corresponding control plants. If plants were challenged with C. *lagenarium* on leaf 2, the enzyme activity at the site of challenge increased in the induced plants prior to rising in the control plants. Large increases in enzyme activity were only evident after the lesions became visible (3-4 days after challenge) in the control plants and this only occurred at the site of challenge. Seven days after challenge, the enzyme activities in the leaf tissue adjacent to the site of inoculation were similar in both the control and induced plants. Correlations were made between peroxidase and chitinase activities induced by several treatments of leaf 1 and the level of protection observed in leaf 2 after challenge with C. *lagenarium.*

Estabrook et al, (1991), have used conserved and non-conserved regions of cDNA clones for phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) isolated from a soybean nodule cDNA library to monitor the expression of members of the two gene families during the early stages of the soybean *Bradyrhizobium japonicum* symbiosis. Their results demonstrated that subsets of the PAL and CHS gene families are specially induced in soybean roots after infection with *B. japonicum*. Furthermore by analyzing a super nodulating mutant line of soybean that differs from the wild type parent in the number of successful infections, the induction of PAL and CHS was shown to be related to post infection events. Nodulated roots formed by a Nod^+ Fix^+ strain of *B. japonicum,* resembling a pathogenic association, displayed induction of another distrinct set of PAL and CHS genes. It was suggested by the authors that symbiosis specific PAL and CHS gene in soybean are not induced by stress or pathogen interaction. Phenylalanine ammonia lyase in activating factor (IF) prepared from chloroplast isolated from sunflower (*Helianthus annus*) leaves was utilized to study its inactivating effect of L-phenyl alanine ammonia lyase from *Rhodotorula glutinis, in vitro*. The effect of inactivation by inactivating factor were compared with those caused by chemicals such as sodium borohydride and nitromethane. The sunflower inactivator acted as an enzyme and enzymatic inactivation caused irreversible loss of PAL catalytic activity accompanied by shortening of the enzyme molecule. However, the capacity of IF inactivated the PAL to bind to L-phenylalanine, the enzyme substrate was maintained. These effect of inactivating factor from sunflower leaves are quite different from those inhibitor isolated from different sources by other marker (Gupta and Creasy, 1991).

Peroxidase and phenylalanine ammonia lyase activities (PAL) was determined in leaves of healthy and inoculated *Brassica napus* cultivars, showing differential disease reaction towards a virulent and a weakly virulent strain of *Leptosphaeria maculans,* the black leg pathogen by Chakraborty *et al* (1993). Both enzymes showed increased activities as a result of inoculation ; PAL activity increasing as early as 12 h after inoculation. The most significant increase in both peroxidase and PAL activity was observed when the moderately resistant cultivar, Cresor, was challanged with the weakly virulent strain. Highest activity of the two enzymes was detected 2d after inoculation. Very low peroxidase activity was detected in both strains of L. *maculans,* while no PAL

activity was detectable in either of the strains. Cytochemical tests revealed increased peroxidase activity following inoculation mainly in the epidermal and guard cell.

Subcellular localization of the pathogenesis - related PR-1 proteins of unknown function was studied in roots of resistant *Nicotiana tabacum* cv. *xanthi* nc uninfected or infected *invitro* by the black root rot fungus *Chalara elegans,* using polyclonal or monoclonal antibodies raised against PR-1a (or b_1) protein (Tahiri-Aloui *et al,* 1993). In healthy tobacco roots, the PR-1 proteins were found to be present in low amounts in intercellular space material, over cell walls and over secondary thickenings of xylem vessels. All these cell compartments were significantly enriched in the PR-1 proteins in infected tobacco root tissues. Cell wall outgrowths, typically induced in hypodermal cells by *Chalara elegans* infection and wall appositions formed in cortical parenchymal cells in response to infection, were also the sites of PR-1 protein accumulation. In contrast, very little occurred in electron - translucent intercellular spaces. PR-1 proteins were also detected in the wall of both inter and intracellular fungal hyphae invading root tissues, but not in axenically cultured *Chalara elegans* or hyphae developing outside roots.

Carver *et al* (1994) reported that seedling leaves of oat cvs Maldwyn and Selma have no known major resistance genes to powdery mildew caused by *Erysiphe graminis* f. sp. *avenae,* but their susceptibility to infection is quantitative. Thus only a portion of fungal germlings successfully overcome cell defences to penetrate host epidermis to form haustoria OH-PAS ([[(2-hydroxyphenyl) amino] sulphinyl] acetic acid, 1,1-dimethyl. ethyl ester) is a potent, specific suicide inhibitor of CAD (cinnamyl alcohol dehydrogenase), an enzyme specifically involved with synthesis of lignin precursors. OH-PAS was shown to inhibit CAD from oat in vitro. For *in vivo* assays of effects on epidermal cell defences, the cut ends of excised seedling leaves were immersed in OH-PAS solution for 24h to allow uptake before inoculation with *E.graminis* conidia. Inoculated leaves were allowed OH-PAS uptake during a further 36 h incubation period. Initial experiments established that OH-PAS at 10^{-3} M decreased the frequency and intensity of localized autofluorescent host epidermal cell responses associated with primary germ tubes (PGTs) and appressoria. Concurrently, OH-PAS treatment doubled the proportion of appresoria forming

haustoria, i.e. it increased quantitative susceptibility by suppressing host cell defences. Similar results were obtained with 10^{-3} M AOPP ($\mathcal{L}$-aminooxy β-phenyl propionic acid), a competitive inhibitor of PAL (phenylalanine ammonia lyase) which catalyzes the first committed step in phenylpropanoid metabolism. Both inhibitors doubled the proportion of appressoria penetrating epidermal cells and forming haustoria. Both inhibitors reduced the frequency and intensity of localized autofluorescent epidermal host cell responses to PGTS and appressoria, although the effect of AOPP was somewhat greater than that of OH-PAS. Neither OH-PAS nor AOPP had any deleterious effects on fungal development. Results support the idea that host autofluorogens accumulating at sites of fungal germ tube contact with epidermal cells are phenolic compounds. In addition the study provides experimental evidence pointing to involvement of products synthesized as part of the lignin biosynthetic pathway in oat epidermal cell defence against attempted penetration by appressoria of E. graminis f.sp. *avenae.*

Seeding leaves of two pairs of near-isogenic barley lines were inoculated with conidia of the powdery mildew fungus, *Erysiphe graminis* D. C. f. sp. *hordei* Marchal, racc 3. by Clark *et al.* (1994). One set of isolines (RISQ 5678 R and RISQ - 5678-S) differed at the ML-O locus where the recessive allele (ml-O) confers a high degree of race non-specific, penetration based and papilla - associated resistance to *E. graminis,* while the dominant allele (Ml-0) allows a proportion of attacking fungal germlings to succeed in infection. The second isoline set (Algerian R and Algerian-5) differed at the Ml-a locus where the dominant allele confers race-specific, epidermal cell death resistance visible only by light microscopy. The recessive allele (Ml-a) allows a proportion of attacking fungal germlings to succeed in infection. Leaf samples were taken at 0,2,4,6,8,10,12,15,18,21 and 24 h after inoculation to examine the timing of host epidermal cell cytoplasmic aggregate responses (visible by light microscopy) relative to phenylalanine ammonia lyase (PAL) mRNA transcript accumulation (determined by quantitative northern blots), and PAL enzyme activity (using radiolabelled phenylalanine). *Erysiphe graminis* produced primary germ tubes (PGTs) within 2h and appressorial germ tubes within 6-10 h of inoculation. In all isolines, host epidermal cell cytoplasmic aggregates fromed and subsequently dispersed beneath PGTs, between 2 and 10 h and beneath appressoria betwen 6 and 15 h. Concurrently, biphasic patterns of PAL transcript accumulation,

typed by plaks at 4 and 12 h occurred in all isolines. Temporal patterns of PAL enzyme activity were roughly similar to those of PAL transcript accumulation. Fungal germ tube contact initiated host epidermal cell cytological responses common to all isolines, induced PAL transcript accumulation, and increased PAL activity regardless of the Mendelian inheritance of "major gene-resistance factors" in the barley isoline sets. Thus there was PAL induction associated with a general defence to infection, but no unusually strong correlation between PAL induction and major gene resistance was found.

Phenylalanine ammonia lyase (PAL) activity in barley leaves was also measured at intervals after inoculation with the powdery mildew pathogen *Erysiphe graminis* f. sp. *hordei.* Measurements were made at times that included the periods of attempted penetration by the *E.* graminis primary germ tube and appressorium. The results demonstrated that extractable enzyme activity increased at 6 h and between 12-15h after inoculation, times consistent with attempted penetration by the primary germtube and the appressorium, respectively. Enzyme activity increased regardless of the resistance or susceptibility of the barley cultivar to the fungus suggesting that the response was non specific and was not a reflection of the resistance or susceptibility of the cultivar to the pathogen prior to the time of penetration. When barley was inoculated with the nonpathogen E. *pisi,* only a single period of elevation in PAL enzyme activity was detected. This was consistent with the fact that, unlike E. *graminis,* E. *pisi* does not produce a primary germ tube. The enzyme activity increased between 9-15h after inoculation, consistent with the time of the attempted penetration of the leaf by the E. *pisi* appressorium. Northern blot analyses to detect the time of appearence of PAL mRNA indicated that the level of the message began to increase at 0.5 h after inoculation with both fungi, and that the intensity of the increase was greatest in response to E *pisi.* The results have been discussed with respect to the presumed importance of host phenolic compound metabolism that occurs as a response to the fungal infection process. (Shiraishi et al, 1995).

In order to determine whether the level of phenylalanine ammonia-lyase (PAL) activity in sorghum mesocotyl tissues is elevated in response to inoculation. Orcyzk *et al* (1996) used sorghum cultivars that differed in their ability to synthesize anthocyanins in response to light. These cultivars provided a means

of distinguishing the light induced high background levels of PAL from increases in PAL that occur as a result of attempted infection. The induction of PAL as a response to both light and to attempted fungal infection was further confirmed by a time course study of RNA blot hybridizations with a barley PAL cDNA. Results showed that PAL transcripts in the cultivar that did not synthesize anthocyanins began to accumulate within 3 h of exposure of the tissue to light but that the level of the transcript decreased rapidly thereafter. However, when the same cultivar was inoculated with the nonpathogen *Bipolaris maydis* the intensity of the PAL transcript remained elevated throughout a period of 24h after inoculation. The results demonstrated that in this monocotyledonous host it is necessary to separate the naturally occuring high levels of PAL activity that are induced by light from the induction of PAL enzyme activity which occurs as a response to attempted fungal infection.

Four phytoalexins were identified from inoculated stems of cocoa genotypes resistant to *Verticillium dahliae.* Following purification by repeated flash chromatography and TLC, they were identified by NMR and GC-MS. The most abandant and polar compound was the triterpenoid arjunolic acid; two related phenolics were 3,4 dihydroxyacetophenone and 4. hydroxy acetophenone. The least polar was unambiguously identified as the most stable form of elemental sulphur, cyclooctasulphur S_8, by Gc- Ms and X-ray crystallography. Respective toxicities to V. *dahliae* conidia (ED 50 germination in $\mu g\ ml^{-1}$) were 12.8, 92.5, 7.2, 3.6. Sulphur and arjunolic acid first appeared after 10 and 3 days respectively, were present in the wood of stems at levels well above (7/ x13) those required for toxicity and they persisted for long (7/ 50 days) periods; they were found only after infection whereas the phenolics were detected in control stems and were enhanced to similar extents by infection or wounding . Sulphur accumulation was localized to xylem cells. In contrast, condensed tannins, although increasing approx. two. fold after infection, were performed, of low toxicity (ED_{50} 7/ 383 $\mu g\ ml^{-1}$) and concentrations in two resistant and one susceptible genotypes were not significantly different. This is the first report of phytoalexins in *T.* cacao and of sulphur accumulation in plants linked with active defence (Resende *et al*; 1996).

Groundnut leaves (cutlivar TMV2) infected with the fungal pathogens, *Cercospora arachidicola, Phaeoisariopsis personata* and *Puccinia arachidis*

accumulated 1830, 664 and 162 nml phytoalexins g^{-1} fresh wt., respectively, 4 weeks after inoculation, whereas leaves infested with *Frankliniella* sp. for the same period contained 1.25 nmol phytoalexins g^{-1} fresh wt. (Rao et al. 1996). Spraying abraded leaves with salicylic acid (0.01M) resulted in the accumulation of 1270 nmol phytoalexins g^{-1} fresh wt. 120 h after treatment and irradiation of abaxial leaf surfaces with U.V. light (254nm) for 48h and incubation in the dark for a further 96h caused the accumulation of 393 nmol phytoalexins g^{-1} fresh wt. Compounds with U.V. spectra corresponding to isoflavanones were almost exclusively synthesized in response to abiotic elicitors but in leaves infected with fungal pathogens formononetin, diadzen, and medicarpin were also present, though as minor components.

Reiss and Bryngelsson (1996) found that the perthotrophic fungus *Drechslera teres,* the causal agent of net blotch disease in barley, induces the accumulation of pathogenesis related (PR) proteins in barley leaves which was demonstrated by isoelectric focusing. The same protein pattern was also found in leaves treated with a toxin extract from the culture filtrate of *D. teres* as well as after infection with *Erysiphe graminis* f.sp. *hordei* or *Puccinia hordei.* Some of the proteins induced by infection with *D. teres* were charecterized as peroxidases, β-1,3- glucanases and chitinases by isoenzyme analysis. Immuno detection following western blots demonstrated that the induced proteins are the same as those that accumulate after inoculation of barley with *E. graminis;* basic PR-1a and b proteins, thoumatin like (TL) proteins, β-1,3 glucanases and chitinases. The accumulation of PR-1 type proteins, chitinases and TL- proteins was analysed quantitatively by ELISA.

Oligonucleotide primers made complementary to conserved sequences in phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) genes previously cloned from other species were used to amplify segments of the corresponding genes from sorghum Dycui et at (1996). Greater than 70% base sequence identity with homologous genes confirmed that a 535 bp clone (PAL 1-1) and a 620 bp clone (CHS2G) were derived from the coding regions of PAL and CHS respectively. When used to probe genomic digests, the two clones detected 3 and 5 Eco RI fragments, respectively. One fragment for each probe was polymorphic in the parents of a mapping population, permitting a locus for each gene to be located on a sorghum RFLP linkage map. RNA gel blot analyses

were performed on extracts from control seedlings and from seedlings challenged with either of two fungal sorghum pathogens or a pathogen of maize that elicits a hypersensitive response in sorghum. Although low levels of PAL and CHS mRNA transcripts were present constitutively in the controls, both rapidly accumulated to high levels following inoculation with the maize pathogen, *Bipolaris maydis,* and decreasing amounts of mRNA were apparent by 120h post inoculation. Since challenge with *Sporisorium reilianum* was by needle inoculation, an additional control of mock inoculations was included for this pathogen. However, no differences in the amount of mRNA for either gene were detected over the sampling period, whether samples from uninoculated, mock inoculated or spore inoculated seedlings were tested. Differences in the timing and level of mRNA accumulation were detected in seedlings after inoculation with *Peronosclerospora sorghi.* While seedlings of both resistant and susceptible cultivars accumulated higher levels of PAL and CHS mRNA than uninoculated controls, the accumulation of mRNA in resistant cutlivars was higher and longer rasting than that in susceptible cutlivars.

Three groups of *Orobanche cumana* seedings were distinguished according to the peroxidase content of the cells in the radicles : (1) those with neither extracellular nor intracellular peroxidase and whose radicles have a smoth apex (these were classified as non-infective); (2) those with a high peroxidase content of the nuclei and the cytoplasm layer adjacent to the cell wall, as well as excretion of peroxidase from the apex of the radicles : (3) those with a similarly high peroxidase activity in the parasite cells, but without extracellular excretion (Antonova and Terborg, 1996). The apices of the radicles of the last two groups are swollen. It has been suggested that these belong to *O.cumana* races C and D respectively. The extracellular peroxidase in *O. cumana* race C reacts with phenolic compounds, which are lignin precursors of the host, resulting in host resistance due to the formation of lignin layers in sunflowers possessing the Or 3 gene for resistance. The absence of extracellular peroxidase in *O.cumana* race D prevents lignin - formation and enables the parasite to attach to the host vascular system. Comparison on these data with the information on the earlier *O. cumana* races A and B, and older sunflower cultivars, points to a crucial role of peroxidase in the process of breeding new sunflower cultivars and the evolution of new *O. cumana* races.

Phytophthora megasperma f.sp. *glycinea* infection greatly induced the synthesis and accumulation of β-1,3 glucanases and chitinases in hypocotyls and leaves of soybean seedings. Native polyacrylamide gel electrophoresis and isoelectric focusing were used to identify and quantify the isoforms of the two hydrolases in soybean tissues. Some β-1,3 glucanase isoforms were constitutively experssed in soybean, but other isoforms only accumulated in soybean or only in a particular organ of soybean after inoculation with *P.m.* f. sp. *glycinea* infection or after treatment with mercuric chloride. The pI 8.4 isoform, Gb2 of β-1,3 glucanase constitutively present in soybean hypocotyls and leaves completely disappeared after mercuric chloride treatment. *P.m.* f. sp. *glycinea* infection and mercuric chloride treatment leads to the accumulation of distinctly different isoforms of chitinase in soybean tissues. The acidic chitinase isoform Ca 4 was induced and accumulated in hypocotyls, but not in leaves, after pathogen infection and mercuric chloride treatment. In general, β-1,3 glucanase and chitinase isoforms accumulated in the diseased soybean tissues during symptom development in both compatible and incompatible interactions. The results also suggested that pathogen infection and chemical stress may function in a different organ specific manner to induce isoforms of β-1, 3 glucanase and chitinase in soybeans (Seung and Byung, 1996).

Accumulation patterns of specific isozymes of pathogenesis related proteins in the resistant tomato genotypes 71B², NCEBR-1, NCEBR-2 and th susceptible cultivar Piedmont following inoculation with *Alternaria soloni* were described by Lawrence et al (1996). Western blot analysis demonstrated that four isozymes of chitinase (26,27, 30 and 32 KDa) were induced in all genotypes upon challenge with A. *solani,* but only resistant lines had significantly higher constitutive levels of the 30 KDa isozyme as well as total chitinase activity. In addition, the 30 kDa chitinase isozyme was found to accumulate to significantly higher levels in resistant lines during pathogenesis than the susceptible genotype. Two isozymes of β-1,3 glucanase (33 and 35 KDa) were detected in all genotypes, but a slightly higher constitutive level was detectable in all resistant lines when compared to the susceptible. Similar accumulation patterns of these isozymes were observed in all genotypes during the course of pathogenesis. Purified preparations of acidic and basic tomato chitinase and β-1,3 glucanase isozymes were tested for their antifungal activity against A. *solani* invitro. Results

presented in this study indicate that only basic isozymes of chitinase and β-1, 3 glucanase were inhibitory to A. *solani* whereas, no inhibitory activity was observed with the acidic isozymes. The results of this study suggest that a higher constitutive level of chitinase and β-1,3 glucanase and the induction pattern of a 30 KDa chitinase isozyme in early blight resistant breeding lines is related to genetically inherited resistance of tomato to A. *solani.*

It was also reported by Wibber et al (1996) that in the interaction between *Cladosporium fulvum* and tomato, resistance against the fungus correlates with early induction of transcription of genes encoding apoplastic chitinase and1,3 β-glucanase and the accumulation of these proteins in inoculated tomato leaves. For vacuolar, basic isoforms of chitinase and 1,3-β glucanase, however, early gene transcript accumulation was observed in both incompatible and compatible interactions. Only temporal differences in gene transcript accumulation were observed for each isoform studied. Expression of the acidic chitinase gene was observed primarily near leaf vascular tissue. Expression of the basic chitinase and the basic and acidic 1, 3-β glucanase genes was less confined to particular tissues. No preferential accumulation of gene transcripts in tissue near penetrating hyphae was observed in compatible or incompatible interaction.

Injection of purified race specific elicitors, AVR4 and AVR9, in tomato genotypes cf4 and cf9, respectively, induced primarily differential expression of acidic chitinase and acidic 1,3-β glucanase. The induction, observed most abundantly in resistant genotypes, correlated well with the difference in gene expression previously observed in time course experiments of compatible and incompatible C. *fulvum* tomato interactions. An acidic β-1,3- glucanase and three isoforms of chitinase (A,B,C) were isolated and purified from cucumber (*Cucumis sativus* L.) leaves inoculated with a necrogenic fungus, *Colletotrichum lagenarium.* Tests of antifungal activity to C. *lagenarium in vitro* were conducted with the purified β-1,3 glucanase, the three purified isoforms of chitinase and with intercellular wash fluids (ICF) from cucumber leaves above those inoculated with C. *lagenarium* or those treated with water. β-1,3 glucanase alone significantly inhibited spore germination at a concentration of 250 $\mu g\ kl^{-1}$ buffer solution and inhibited the growth of C. *lagenarium* at a concentration of about 7 μgml^{-1} agar medium. The latter concentration is equivalent to the concentration in infected cucumber leaves 7 days after inoculation with *C. lagenarium* and in

leaf 2 of induced plants 3 days after challenge with the fungus. Chitinase A and B did not inhibit spore germination or fungal growth at the concentrations tested, whereas chitinase C inhibited spore germination only at 1 mg ml^{-1} and inhibited fungal growth at 66 μg ml^{-1} agar medium, the highest concentration tested. β-1,3 glucanase in combination with chitinase C inhibited fungal growth synergistically. Lysis of hyphal tips and abnormal growth were observed in the presence of a mixture of β-1,3 glucanase and chitinase C. Intercellular wash fluid (ICF) and concentrated ICF (5x) from control and induced plants did not inhibit fungal growth. However, the crude ICF in the presence of purified β-1,3 glucanase and chitinase C had antifungal activity (Ji and kuc, 1996).

Generation of superoxide anion [0-2(0)] and peroxidase activity were significantly increased in bean leaves infected with incompatible and compatible pathogens : *Botrytis fabae* and *Botrytis cinerea,* respectively, but the induction was greater on direct inoculation with B. *fabale,* than with *B. cinerea* as determined by Urbanek et al (1996). A slightly higher 0-2(-.) level was also detected in the parts of leaves surrounding the inoculation side. Overproduction of 0-2(-.) was observed earlier than the increase in peroxidase activity. Pretreatment of the leaves with methyl jasmonate enhanced both 0-2(-.) production and peroxidase activity following inoculation with B. *cinerea.* Induction of superoxide dismutase activity after the infection was less pronounced than changes in 0-2 (-.) level. The differences in the rate of NADH oxidation in the extracts from control and inoculated leaves, correlated with the differences in the rate of 0-2 (-.) production. The results indicate that 0-2 (-.) level is one of the essential factors responsible for the difference in the interactions between bean plant and compatible and incompatible pathogens.

Tobacco plants (*Nicotiana benthamiana L.*) have been transformed with a T-DNA vector construct carrying the cDNA pBH6-301, encoding the major pathogen induced leaf peroxidase (Prx8) of barley, under control of an enhanced CaMV 356 promoter. Progeny from three independent transformants were analyzed genetically, phenotypically and biochemically. The T-DNA was steadily inherited through three generations. The barley peroxidase is expressed and stored to the intercellular space in the transgenic tobacco plants. The peroxidase can be extracted from the intercellular space in two molecular forms from both barley and transgenic tobacco. The tobacco expressed forms are

indistinguishable from the barley expressed forms as determined by analytical isoelectric focusing (PI 8.5) and western blotting. Staining for N-glycosylation showed that one form only was glycosylated. The N-terminus of purified Prx8 from transgenic tobacco was blacked by pyroglutamate, after the removal of which, N-terminal sequencing verified the transit signal peptide cleavage site deduced from the cDNA sequence. Phenotype comparisons show that the constitutive expression of Prx8 lead to growth retardation. However, an infection assay with the tobacco powdery mildew pathogen *Erysiphe cichoracearum* did not indicate that the transgenic plants had achieved enhanced resistance. (Kristensen *et al, 1997).*

Serological relationship between host and Pathogen

It is now well established that immune system functionally similar to that of animals exists in plants. The serological cross reactivity between host and pathogen has been a subject of considerable interest to a number of workers and a number of reviews pertaining to this area have been published previously (DeVay and Adler, 1976; Clark, 1981; Chakraborty, 1988, Purkayastha, 1994). Detection of plant pathogenic fungi within host tissues by serological means is a relatively recent development and a number of recent reviews have been published by workers along this line (Hansen and Wick, 1993; Werres & Steffens 1994).

Conidia of F. *oxysporum* f. sp. *vasinfectum* was reported to contain an antigen that cross reacted with antiserum to cotton root tissue antigens (Charudattan and Devay, 1981). In agar gel double diffusion tests, one precipitin band was formed when antiserum to cotton antigens was reacted with crude fungal antigens, or when antiserum to crude fungal antigens was reacted with cotton antigens. The cross reactive antigen from fungal conidia (CRA) was isolated, purified and partially characterized. The CRA migrated as a single band in polyacrylamide or agar gel electrophoresis, and sedimented as a single band during analytical ultracentrifugation. It was antigenic in rabbits and was a protein carbohydrate complex. The major cross-reactive antigenic substance (CRA), common to cotton (*Gossypium hirsutum)* and certain fungal parasites of cotton roots, was further purified to homogeneity from conidial cultures of F. *oxysporum* f. sp. *vasinfectum (Devay et al., 1981).* Agar gel double diffusion tests indicated

the presence of CRA not only in *F. oxysporum* f. sp. *vasinfectum* and in cotton roots and seed but also in *Thielaviopsis basicola*. Indirect staining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross sections of roots, cut near or just below the root hair zone, the CRA was concentrated mainly around xylem elements, the endodermis and epidermis cells and was present throughout the cortex tissue. Protoplasts prepared from cross sections of young cotton roots also contained the CRA which was concentrated in the region of the plasmalemma. Treatment of conidia and mycelia of F. *oxysporum* f. sp. *vasinfectum.* with antiserum to cotton and using indirect staining with FITC indicated that the CRA was mainly present in hyphal tips and in patch like areas on conidia.

Johnson *et at.* (1982) used antiserum prepared to homogenates of washed *Epichloe typhina* mycelium grown in a liquid medium in an enzyme - linked immunosorbent assay (ELISA) to detect antigens of the fungus in tall fescue (*Festuca arundinacea)* tissue samples. Very low concentrations (100μg/ l) of freeze dried E. *typhina* mycelium could be detected and the pathogen could also be detected in individual seeds of tall fescue. Of 14 fungal genera tested, including *Acremonium, Claviecps, Helminthosporium, Pythium, Rhizoctonia* and *Sclerotium* all showed reactivities less than 0.1% that of *E. typhina*. Cross reactive antigen shared between soybean cultivars and *Macrophomina phaseolina* causing charcoal rot disease was detected. Rabbit antisera were raised against root antigens of soybean cultivars (Soymax and UPSM-19) and *M. phaseolina* isolate (M.P.1) and tested against homologous and heterologous antigens following immunodiffusion tests. When antiserum of M. *phaseolina* was reacted against its own antigens and antigens of susceptible soybean cultivars (Soymax, R-184) strong precipitation reactions were observed. In case of resistant cultivars (UPSM-19 andDS-73-16) no such reactions were observed. Reciprocal cross reactions between antiserum of the resistant cultivars and antigens of three isolates of *M.phaseolina* also failed to develop even weak precipitation bands. Four antigenic substances were found to be common between the susceptible soybean cultivars and isolates of M. *phaseolina* in immunoelectrophoretic tests, but no common antigens were

detected between resistant cultivars and the fungus. Purkayastha and Chakraborty (1983) further detected that in susceptible soybean plants (cvs. Soymax and R-184) a close relationship exists between lower production of glyceollin and presence of common antigens. The production of glyceollin was much higher in resistant soybean cultivars (cvs. UPSM-19 and DS-73-16) where common antigens were absent (Chakraborty and Purkayastha, 1983). Alba *et al.*, (1983) also detected common antigens in extracts of *Hemileia vastatrix* urediniospores and of *Coffea arabica* leaves and roots Antisera were made to both a whole cell and cell wall preparation of *Eutypa armeniacae.* Rhodamine isothiocyanate (RITC) conjugated antisera were tested for reactivity with various fungi on glass slides by Gendloff *et el* (1983). Both antisera showed low specificity, but specificity was improved by cross - adsorption of the RITC. conjugated cell wall antiserum with *Phomopsis viticola.* Woody cross sections from concord grapevines inoculated with E. *armeniacae* and also inhabited by various other fungi were stained directly with the conjugated anti - *Eutypa* rabbit serum. In an indirect staining procedure, sections were treated with anti-*Eutypa* rabbit gamma globulin. Both procedures specifically stained hyphae in wood sections. Hyphae stained indirectly in woody vine sections showed a much brighter fluorescence than analogous hyphae stained by the direct method. Fungi of some species that reacted strongly with the direct method on glass slides did not always react when stained indirectly in woody vine sections in which they were growing.

Indirect immunofluorescence performed by using antisera to culture filtrate molecules of *Phaseolus schweinitzii* has been used to demonstrate the presence of mycelium, and on occasions chlamydospores, in naturally and artificially infested soil samples. The authors could thus identify the kind of progagule most likely to be the source of field isolates of the organisms; this information, which could not be obtained by using selective media, strongly suggested that the pathogen could survive saprophytically in the soil. In contrast, isolated mycelial cell wall preparations did not prove to be a suitable source of immunogenic material for these studies (Dewey *et al, 1984).*

Agnelon and Dunez (1984) used double antibody sandwich ELISA and indirect ELISA technique for the detection of *Phoma exigua* in inoculated tubers and sprouts and in stems grown from these tubers. The fungus was detected in

these different tissues with var- *foveata* being more aggressive, demonstrating the applicability and sensitivity of the techniques. The antibodies produced to the two varieties of the fungus were not specific to their own varieties. They also reacted with *Phoma tracheiphila* but did not react with several other common potato pathogens.

Reddy and Ananthanarayanan (1984) investigated the presence of *Ganoderma lucidum* in betelnut by the fluorescent antibody technique. Presence of *Selerotinia sclerotiorum* in sunflower was also detected by Walcz *et al,* (1989) by enzyme - linked immuno sorbent assay (ELISA).

Immunodiffusion, immunoelectrophoretic and crossed immuno-electrophoretic analyses of rice antigens in relation to sheath rot disease revealed a serological relationship between susceptible rice cultivars and isolates of the causal organism of sheath rot, *Acrocylindrium oryzae*. One precipitin band was observed when the antigen preparation of A *oryzae* was cross-reacted with its own antiserum or against the antisera of four susceptible rice cultivars. No precipitin band was detected between the antiserum of the resistant cv. Mahsuri and antigen preparations from three isolates of A. *oryzae* or between the antigens of the resistant cultivars Mahsuri and Rupsail and the antiserum of *A. oryzae*. Crossed immunoelectrophoretic tests confirmed that there was a common antigen between Mahsuri and Jaya, and between Mahsuri and CR-126-42-1. The precipitin band between the antigen preparation of Jaya and A. *oryzae* was found to be similar (Purkayastha and Ghosal, 1985). The common antigenic relationship between soybean cultivars and *Colletotrichum dematium* var. *truncata* was also ascertained by Purkayastha and Banerjee (1986) following immunodiffusion, immunoelectrophoretic and crossed immunoelectrophoretic tests. At least one antigen was found to be common between host cultivar and the pathogen. No antigenic relationship was observed either between soybean cultivars and the non pathogen (*C. corchori*) or avirulent pathogen (*C. dematium*). Alba and Devay (1985) detected cross reactive antigens in crude preparations and in purified preparations from mycelia of *Phytophthora infestans* Race-4 and Race -1, 2, 3, 4, 7 with antisera for potatoes cv. King Edward and cv. Pentland Dell by using an indirect ELISA technique. It was suggested that the fungal mycelia do not easily release cross reactive antigens into synthetic media where they grow; that most of *P. infestans* cross

reactive antigens were thermolabile and that they could be concentrated by precipitation in the presence of 40% saturated ammonium sulphate (SAS). An antigenic disparity was observed when 40% SAS from P. *infestans* Race-4, mycelial preparation was assayed with antisera for King Edward and Pentland Dell.

Sections of leaves of *Nicotiana tabecum* infected with *Peronospora hyoscyami* f. sp. *tabacina* and of *Erythronium americanum* infected with *Ustilago heufleri* treated with an antiserum directed against the fimbriae of *U. violacea* Fuckel and other fungi were then treated with protein. A - gold complexes to detect the presence and location of fimbrial antigens following transmission or scanning electron microscopy. The infected leaf sections were heavily labelled with gold particles indicating the presence of fimbrial antigens, whereas the control preparations showed only a low background level of labelling. Gold particles were detected on the sections of hyphae, on haustoria and on the nearby plant cells. The intensity of labelling was much higher for P. *hyoscyami* f. sp. *tabacina* than for *U. heufleri* and was particularly high in the walls of the former species. Relatively high levels of labelling occurred over the cells of infected host tissues but little or no labelling occurred over the cells of uninfected host tissues or of the infected host tissues treated with a range of serological controls. This level of labelling was not associated with specific host structures in P. *hyascyami,* but was frequently associated with the chloroplast in U. *heufleri.* The antigens detected inside the host plant cells appeared to indicate that fungal fimbrial protein, either as polymerised fibrils or as isolated sub-units, could penetrate the host plasma membrane and therefore entered the host cytoplasm. (Day *et al., 1986).* Hyphae of *Verticillium dahliae* were detected in cotton root tissue with an ELISA by Gerik *et al.* (1987). A soluble protein extract of V.*dahliae* was used to prepare a specific rabbit antiserum. The reaction of this rabbit antibody to the hyphae of V. *dahliae* was detected with an alkaline phosphatase antirabbit IgG conjugate that hydrolyzed the substrate, napthol - As- phosphate, to a product that reacted with a diazonium salt, yielding a colored precipitate outlining the fungal hyphae. The hyphae were readily observed on and in the root cortex of the host using a dissecting microscope.

Purkayastha and Ghosal (1987) also compared the antigenic

preparations from two isolates of *Macrophomina phaseolina*, a pathogen of groundnut, four non-pathogens of groundnut (viz., *Corticium sasakii, Colletotrichum lindemuthianum* C. *corhori,* and *Botrytis allii)* and five cultivars of *Arachis hypogea* by immunoserological techniques. Common antigens were found among the susceptible cultivars of groundnut and two isolates of M.*phaseolina,* but not between nonpathogens and groundnut cultivars. No antigenic similarity was found between nonpathogens and *M. phaseolina* isolates. Cross immunoelectrophoretic tests confirmed that at least one antigen was common between cv. J-11 and cv. TMV-2, cv. Kadiri 71-1 and cv. TMV-2 and cv. Kadiri 71-1 and isolates of *M.phaseolina.*

Further, changes in antigenic patterns after chemical induction of resistance in susceptible soybean cultivar (soymax) to *Macrophomina phaseolina* was determined by Chakraborty and Purkayastha (1987). Sodium azide (100 μg / ml) altered antigenic patterns in susceptible cultivar (soymax) and reduced charcoal rot disease. Similar results were also obtained by Ghosal and Purkayastha (1987) in susceptible rice cultivar (Jaya) and *Sarocladium oryzae* after altering disease reaction (sheath rot) by the application of gibberellic acid and sodium azide (100 μg / ml). Gerik and Huisman (1988) observed that colony density of *V. dahliae* increased with distance from the root tip, with the maximal density occurring more than 1 cm from the root apex. Colony densities at distances more than 1cm from the tip were relatively constant. The mean colony length of *V. dahliae* was 7.3mm, and increased colony length was correlated with distance from root apices. Hyphae of V. *dahliae* were present through the entire depth of the cortex, and were greatest in the interior of the root cortex at the surface of the vascular cylinder. The colony appearence was consistent with growth of hyphae from the root surface toward the stele. Hyphae of *V. dahliae* also were found within numerous cortical cells. Colonies of *Fusarium oxysporum* similarly stained, were found to be mostly confined to the root surface and the outer cortex.

In enzyme linked immunosorbent assay, antiserum raised against pooled mycelial suspensions from five isolates (designated pf 1, pf2, pf3, pf 10 and pf 11) of *Phytophthora fragariae* detected homologous soluble antigens at protein concentrations as low as 2 μg/ ml (Mohan, 1988). Fungal antigens could also be detected in extracts of strawberry plants infected with P.

fragariae. Root extracts prepared from the alpine strawberry *Fragaria vesca* and *F. ananassa* cv. Cambridge Favourite infected with any of the five isolates studied produced strong reactions in ELISA. In F. *vesca,* ELISA positive material was detectable 6-8 days after inoculation before macroscopic symptoms appeared. The cultivar Red Gauntlet, resistant to pf1, 2 and 3 but susceptible to pf 10 and 11, reflected this differential response in ELISA : the absorbance produced by extracts of plants infected with virulent isolates was significantly higher than that obtained with the corresponding extracts of plants inoculated with avirulent isolates.

An agri-diagnostic *Phytophthora* multiwell ELISA kit, developed for detection of *Phytophthora* in plant tissue, also readily detected *Phytophthora* in soil where soybeans were damaged by P. *megasperma* f. sp. *glycinea* (Pmg) (Schmitthenner, 1988). Only low level of *Phytophthora* were detected in soil stored at 3°C. Following cold storage, high levels of *Phytophthora* could be detected directly from soil, after *Phytophthora* damping off of soybean seedlings was induced. But low *Phytophthora* detection was obtained from soybean leaf discs floated on water over infested soil for 24 hrs. Pmg was the only *Phytophthora* isolated from such leaf discs using selective media. It was concluded that *Phytophthora* was detected best with an ELISA test of soil with actively rotting roots or from leaf disc baits with actively growing mycelium.

Amouzon *et al.*, (1988) reported that the antiserum obtained by injecting robbits with mycelial protein extracts of one strain of *Phytophthora fragariae* had a dilution end point of 1:64 in double diffusion and 1/512,000 in indirect ELISA. This serum could detect 11 different strains of P. *fragariae* in pure culture and the pathogen in naturally infected or inoculated roots. Although the sensitivities of direct DAS and indirect ELISA were comparable, th direct DAS-ELISA was more specific for the detection of *P. fragariae* in strawberry roots. The antiserum failed to react with 18 fungal species isolated from underground parts of strawberry but reacted with some strains of *P cactorum,* which parasitized only rhizomes but not roots, and *Pythium middletonii,* which was isolated sometimes in association with *P. fragariae* from strawberry roots. In inoculated strawberry roots, *P. fragariae* was detected reliably by ELISA several days before oospores were found and before symptoms developed. Thus direct DAS.ELISA may be useful for early detection of infection and for the detection of latent infections of strawberry plants by P. *fragariae.*

Resting spores of *Plasmodiophora brassicae* from soil and root were detected by indirect fluorescent antibody technique. Infested soil and roots were stained the fluorescent antibody technique with the IgG and FITC conjugated antirabbit IgG-Sheep IgG. Resting spores were effectively detected and also clearly differentiated from small particles of soil and tissues of plant in the reflected light fluorescence microscope(Arie *et al.* 1988). An indirect ELISA for quantitative detection of *P. herpotrichoides* infections in wheat was developed by Unger and Wolf (1988). All tested isolates of the virulent varieties P. *herpotrichoides* var. *herpotrichoides,* P. *herpotrichoides* var. *acuformis* or the W - and R-type reacted on a high level in th test, while the less virulent *P. angnioides* was assessed only with 40% and the avirulent *P. aestiva* with 20% of the homologous reaction. No cross reactions occurred with extracts of 11 other species of *in vitro* cultivated fungi nor with plant material infected with other pathogens. The infection profile throughout the leaf sheaths was clearly reflected by ELISA. The examination of 24 stem base samples from the field showed that the values assessed by ELISA correlated well also with the disease indices of naturally infected plant material.

Antigens prepared from two resistant cultivars (UPSM-19 and DS 73-16) and two susceptible cultivars (DS-74-24-2 and PK-327) of soybean and three strains of *Myrothecium roridum* (M-1, ITCC-1143 and ITCC-1409), a causal organism of leaf spot disease were tested against antisera of pathogen. Immuno-diffusion tests revealed that common antigens were present only between the virulent strain and susceptible host cultivars. But no such cross reactive antigen was detected in case of resistant cultivars (UPSM-73-16). Immunoelectrophoretic analysis showed that one common antigen was shared by rocket immunoelectrophoresis (Ghosh and Purkayastha, 1990). Cross reactive antigens (CRA) were also detected between susceptible soybean cultivars and the virulent strain of *Colletotrichum dematium* var. *truncata* but CRA could not be detected between soybean cultivars and an avirulent strain of C. *dematium* or non-pathogen (*C. corchori*). Results of immuno diffusion and immunoelectrophresis showed absence of common antigen between resistant cv. UPSM-19 and the pathogen, while the results of indirect ELISA indicated the presence of common antigen between the two at a very low level. Alteration in antigenic patterns of soybean leaves after induction of resistance by the treatment of cloxacillin (100μg / ml) were further detected by Purkayastha and Banerjee (1990).

Watabe (1990) reported on the usefulness of immunofluorescent antibody technique for detection of *Phytophthora* in soil. Autofluorescence and the nonspecific staining of soil particles interfered with the detection of the fungi in soil. Pretreatment of the samples with gelatin rhodamine conjugate prevented the samples from the autofluorescence and the nonspecific staining and therefore permitted the immunofluorescent antibody staining in soil. Stained *Phytophthora* was easily detected on the yellow orange background.

A polyclonal antiserum, prepared in a rabbit immunized with a mycelium extract of *Phytophthora infestans* reacted in an enzyme linked immunosorbent assay (ELISA) with mycelial extracts of two *Phytophthora* species but not with those of 10 other microorganisms found on potato. P. *infestans* mycelium in leaf tissue was readily detected by ELISA using either the plate - trapped antigen or F (ab^1) antibody fragment techniques. The amount of mycelium in leaf extracts was estimated by comparing the values obtained in ELISA with those for known concentrations of P. *infestans* mycelium (Harrison *et al., 1990)* Ricker *et al. (1991)* detected water solube antigens produced by *Botrytis cinerea* in picked and naturaly infected grape juice by using an enzyme immunoassay with an indirect format of antibody HRPO conjugated bound to polyclonal rabbit antibodies directed against *B. cinerea* (anti BcIgG). Protein A purified γ globulin from an early bled antiserum (803.7), which reacted primarily with low molecular weight compounds present only in extracts of B. *cineria,* was used to specifically detect *B.cinerea* and quantify levels of infection in juice from infected grape berries. Late bled higher titre antiserum (803-19), which cross reacted with proteins and carbohydrates present in extracts from species of *Botrytis, Aspergillus, Penicillium* and *Uncinula,* was used ot quantify the levels of rot caused by presence of multiple fungi. Minimum detectable levels of infections, based on mixture of clean and infected juice, were.25-.5% with (803-7)/IgG, and 0.2% with 803-19 IgG. Cross reactivity of anti BeIgG with extracted antigens (water soluble) from sterile and reproductive strains of several fungi was negligible in early bled antiserum and increased in subsequent collections. The increase in cross reactivity in late bled antiserum corresponded with an increase in the overall serum titres for anti BeIgG to antigens from *B. cinerea.*

Polyclonal antisera prepared against purified mycelium proteins from *Verticillium dahliae,* the predominant fungus species in the potato early drying

complex was tested against crude mycelial preparations of *Verticillium* spp. using indirect ELISA (Sundaram, 1991). It reacted positively with 11 to 12 V. *dahliae* isolates from potato, cotton and soil but negatively with one isolate from tomato. The antisera did not react with mycelial proteins from *Fusarium* spp. from potato and cotton, with a *Colletotrichum* sp. from potato or with a isolate of *Rhizoctonia solani* from sugarbeat. Double antibody sandwich ELISA, using polyclonal antisera, detected V. *dahliae* and *V.allo-atrum* in infected roots and stems of potato.Benson (1991) compared two commercial serological assay kits to a culture plate method for detection of *Phytophthora cinnamomi* in root samples from infected azaleas. Both the multiwell E Kit and the rapid assay F kit detected. *P. cinnamomi* on azalea roots beginning 1 week after inoculation. Agreement between immunoassay kits and culture plate results for detection of *P. cinnamomi* was not consistent beginning 3-5 week after inoculation. Root symptoms, but not foliar symptoms, of *Phytophthora* root rot were evident during this period. There was a positive correlation between root rot severity in green house trials and root sample absorbance (multiwell) or meter reading (rapid assay) but not between symptom severity and immunoassay results. The multiwell kit detected *P. cinnamomi* in root samples containing as little as 1.0% infected root tissue. In a commercial nursery survey, 5, and 15% of the azalea root samples of two nurseries had positive ELISA values that were unconfirmed by culture plate. The rapid assay kit detected *P. cinnamomi*, was easy to use, and gave results in a short time.

Methods for sampling turf grass tissue were compared for their effectiveness in monitoring *Pythium* blight epidermis with enzyme linked immunosorbent assay (ELISA). Samples consisted of whole plants picked by hand and assayed as whole plants; whole plants sectioned into lower, middle and upper strata components; leaf clippings collected with a red mower set at a 1.2cm cutting height. ELISA readings for mowed samples generally matched those for whole plucked samples. Fluctuations in detectable *Pythium* antigens were most pronounced on the uppermost stratum compared with moderate to very little change in ELISA readings for the two lower strata. Several episodes of *Pythium* antigen increase were detected by ELISA assays of mowed samples, although signs and symptoms of *Pythium* blight were not evident. However, increase in ELISA readings for *Pythium* coincided with, but did not generally

precede, the onset of blight symptoms with a 2 to 3 day sampling interval. Shane (1991) concluded that antibody aided detections was useful for verification of diagnosis and determination of general *Pythium* population fluctuations, but was not satisfactory for advanced detection of blight epidemics.

Results of conventional isolation techniques for *Pythium violae* were compared with the assay of cavity spot lesions using polyclonal antibodies raised to *P. violae or P. sulcatum,* in competition ELISA (Lyons and White, 1992). Where lesions were artificially induced the test confirmed which pathogen was causal. With cavities developed on the field grown carrots *P. violae* again predominated and the ELISA confirmed this. In one sample *P. sulcatum* was also isolated from a small number of lesions and was not detected in ELISA. The competition ELISA did not indicate presence of either *Pythium* in a range of non-cavity spot lesions from which attempts at isolation were also negative. Linfield (1993) elicited polyclonal antiserum against a strain of *Fusarium oxysporum* f. sp. *narcissi* GCRI 80/26) and a specific and sensitive enzyme linked immunosorbent assay was developed. Antiserum raised to cell wall fractions gave better recognition than that to cytoplasmic fractions. Recognition was equally good in artificially and naturally infected bulbs. Little cross reactivity in bulb tissue was shown by three other bulb-rotting fungi. Nine isolates of *F. oxysporum* f. sp. *narcissi* from a wide geographic area gave similar results in an indirect ELISA of mycelial extracts, although some crossreactivity was observed with two other *Fusarium* spp. Four *Fusarium* spp and four other fungi showed little cross reactivity. Ten days after inoculation the pathogen was readily detected in the base plate area of three *Narcissus* cultivars and points remote from the inoculation site in the most susceptible cultivar. A direct correlation was observed between positive results in the enzyme linked immunosorbent assay and recovery of pathogen on selective medium.

Polyclonal antibodies produced against culture filtrate and mycelial extracts immunogen preparations from the soybean (*Glycine max*) fungal pathogen *Phomopsis longicolla* were purified to the immunoglobulin fraction and tested in indirect enzyme linked immunosorbent assay (ELISA) and in double antibody sandwich ELISA (DAS-ELISA) (Brill et al, 1994). The PABs raised to culture filtrate were more specific but less active in binding to members of the *Diaporthe. Phomopsis* complex than were those raised to the mycelial

extract immunogen preparation. DAS-ELISA was more specific and 100-fold more sensitive in detecting members of the complex than was indirect ELISA. Variability in specificity between different PABs was lower in DAS-ELISA compared with indirect ELISA. Immunization of one rabbit with culture filtrate over an extended time resulted in maximum anti *P longicolla* activity after three immunization, and the activity became constant against most members of the complex at the same time. Reactivity to some culture of *P. longicolla* was undetectable following the fourth and fifth immunization, where as reactivity to all of other cultures of the complex remained high. Polyclonal mouse ascite antibodies were raised against soluble protein extracts of chlamydospores and mycelium. The IgG fraction was purified and biotin labelled to device a fungal capture sandwich ELISA. ELISA detected both brown and grey cultural types of T. *basicola* and had negligible corss reactivity with other soil borne fungi. The minimum detection limit of ELISA was between 1 and 20 ng of T. *basicola* protein depending on the assay. T. *basicola* could be detected in cotton roots two days after inoculation. At this time, initial symptoms was apparent. The antibody also was used to observe T. *basicola* on cotton roots with immunofluoresecnce microscopy (Holtz *el al.*, 1994).

Purkayastha and Pradhan (1994) observed that three strains of *Sclerotium rolfsii* were serologically different and their pathogenecities also differed markedly with host cultivars. Virulent strains 266 and 23 showed common antigenic relationship with their respective susceptible host cultivars but not resistant cultivars. Antigenic change in a susceptible cv. AK-12-24 after treatment with a systemic fungicide kitazin was also evident. They suggested that resistance could be induced in susceptible plants if specific antigens were eliminated by suitable treatment. Antigens obtained from six Tocklai varieties of tea, four isolates of *Bipolaris carbonum* and non-pathogens of tea (*Bipolaris tetramera* and *Bipolaris setarae),* were compared by immunodiffusion, immunoelectrophoresis and indirect ELISA in order to detect cross reactive antigens (CRA) shared by the host and the parasite. CRA were found among the susceptible varieties (TV-9,17 and 18) and isolates of *B. carbonum* (BC 1, 2, 3 and 4). Such antigens were not detected between isolates of *B. carbonum* and resistant varieties (TV-16, 25 and 26), non-pathogens and tea varieties, as well as non pathogens and isolates of *B. carbonum.* Indirect staining of

antibodies using fluorescein isothiocyanate (FITC) indicated that in cross sections of leaves (TV-18), the CRA were concentrated mainly around epidermal cells. Treatment of mycelia and conidia of *B. carbonum* with antisera to leaves (TV-18) and indirect staining with FITC indicated the presence of CRA in the young growing hyphal tips and conidia, (Chakraborty and Saha, 1994).

A serological test was developed by Jamanx and Spire (1994) that allows the early detection of infection of young petals by *Sclerotinia sclerotiorum* an important pathogen of rapeseed. Soluble mycelial extracts of *S. sclerotiorum* were used to produce the first generation antiserum. This was not specific for *S. sclerotiorum* in double DAS ELISA and allowed the screening of cross reacting fungal species such as *Botrytis cinerea,* a pathogen commonly present on rapeseed petals Using a polyclonal anti *B. cinerea* serum enabled the adsorption by serial cycles, of *S. sclerotiorum* antigens commonly to B-*cinerea* Residual antigens were used as immunogens for the production of two second generation antisera (S1 and S2) which were then tested by DAS-ELISA. Cross reactions with *B.cinerea* decreased with purification cycles of the immunogen whereas cross-reactions with some unrelated fungi slightly increased. S. *sclerotiorum* and *B. cinerea* were distinguishable using antiserum S2.

Among the 12 varieties of tea tested against three isolates of *Pestalotiopsis theae* , causal agent of grey blight disease, Teen Ali - 17/1/54 and TV-23 were found to be highly susceptible while CP-1 and TV-26 were resistant under identical conditions. Leaf antigens were prepared from all the tea varieties, three isolates of P. theae and a non pathogen of tea (*Biplaris tetramera).* Polyclonal antisera were raised against mycelial suspensions of P. *theae* (isolate Pt.2) and leaf antigens of Teen Ali-17/1/54 and CP-1. These were compared by an immunodiffusion test and enzyme linked immunosorbent assay to detect cross reactive antigens (CRA) shared by the host and the parasite. CRA were found among the susceptible varieties and isolates of P. *theae* (Pt-2 and 3). Such antigens were not detected between isolates of P. *theae* and resistant varieties, *B. tetramera* and tea varieties or isolates of P. *theae.* Indirect staning of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross sections of tea leaves the CRA was concentrated in the epidermal cells and mesophyll tissues. CRA was present in the young hyphal tips of the mycelia and on the setulae and appendages of the conidia of *P. theae* (Chakraborty *et al., 1995).*

Wakeham and White (1996) raised polyclonal antisera against whole (coded : 16/2), and sonicated (coded :15/2) resting spores of *Plasmodiophora brassicae* as well as soluble components prepared by filfration and ultracentrifugation (coded : SF/2). Cross reactivity of all three antisera with a range of soil fungi, including *Spongospora subterranea* was low. Test formats including Western blotting, dip-stick, dot blot, indirect enzyme linked immunosorbent assay (ELISA) and indirect immunofluorescence were assessed for their potential to detect resting spores of *P. brassicae* in soil. Dot blot was least sensitive, with a limit of detection level of $1x10^7$ resting spores g^{-1} in soil. With Western blotting the lower limit of detection with antiserum 15/2 was $1x10^5$. This antiserum showed the greatest sensitivity in a dip-stick assay, indirect ELISA and indirect immunofluorescence, for all of which there was a limit of detection of $1x10^2$. The indirect ELISA was successful only after the substitution of alkaline phosphatase by protein. A conjugated horseradish peroxidase. Of the assays tested, indirect immunofluorescence appears to be the most rapid and amenable assay for the detection in soil of low levels of resting spores of *P. brassicae.*

Polyclonal antiserum was raised against mycelial extract of *Pestalotiopsis theae* and immunoglobulin fractions were purified by ammonium sulfate fractionation and chromatography on DEAE sephadex. In ELISA, antiserum diluted upto 1.16000 detected homologous antigen at 5 mg / L concentration and at 1:125 antiserum dilution fungal antigens could be detected at concentration as low as 25 μg / L. In 15 varieties of tea tested, from Darjeeling, UPASI and Tocklai Research stations, absorbance values of infected leaf extracts were significantly higher than those of healthy extracts at 40 mg/L concentration in indirect ELISA. ELISA positive material was detected in tea leaves as early as 12h after inoculation with P. *theae.* At antiserum dilution upto 1 : 125, pathogen could be detected in infected leaf extracts upto 2mg / L concentration. The results demonstrated that ELISA can be used for early detection of P. *theae* in leaf tissues even at very low level of infection (Chakraborty *et al., 1996).*

Two monoclonal antibodies (MAbs) were produced against soluble antigens from the 'Ascochyta complex' fungi by Bowen *et al (1996).* Specificity of MAbs was tested by ELISA using antigen coated wells. MAbs secreted by

the monoclonal hybridoma cell line JIM 44 recognized epitopes present in the antigen preparations from *Mycosphaerella pinodes* and *Phoma medicaginis* var. *pinodella*, but not those present in preparations from *Ascochyta pisi*. At high tissue culture supernatent concentration, MAbs produced by the monoclonal line JIM 45 recognized epitopes from all three fungi, however, on dilution of MAb the antigens from A. *pisi* were recognized preferentially to those from *M.* pinodes and P. *medicaginis* var. *pinodella*. On the basisof heat and periodate treatment of the antigens from the three fungi it can be concluded that the epitope recognized by JIM 44 is carbohydrate in nature, whereas that recognized by JIM 45 is proteinaceous in nature, carried on a glycoprotein antigen. Antigen preparations from other fungi, including other pea pathogens, non pathogens associated with pea and other fungi closely related to the 'Ascochyta complex.' were not detected with either of the two MAbs. Antigen preparations from peas could be used to differentiate healthy and infected seeds in a dot-blot assay, therefore indicating the potential of using the MAbs in the development of a diagnostic test for infection of *Pisum* seeds by the 'Ascochyta complex' fungi.

A polyclonal antiserum was raised against spore balls of *Spongospora subterranea* f.sp *s*ubterranea by Walsh *et al (1996)* to detect as little as 0.02 spore balls in an enzyme linked immunosorbent assay (ELISA). In spiked soil samples, the antiserum detected 100 spore balls per g soil. However, the different spore ball contamination levels were discriminated better in ELISA tests at concentrations above 2000 spore balls per g soil than at lower concentrations. In contrast, a bioassay test based on baiting soils with tomato seedlings gave good discrimination of spore ball contamination levels in spiked soils containing <1000 spore balls per g soil and poor discrimination of levels in spiked soils containing >2000 spore balls per g soil. Tests on a limited number of field soils suggested ELISA may be capable of predicting disease levels on tubers grown is such soils better than the bioassay. The antiserum did not react with 30 other microorganisms tested, including many that are saprophytes or pathogens on potatoes and resting spores of the taxonomically related *Plasmodiophora brassicae.* It detected spore balls of different cultivar origin equally well. It also detected spores from different geographical origins. An attemp to improve the sensitivity of the serological detection through

concentrating spore balls from field soils by sieving was unsuccessful. Cross absorption of the antiserum with uncontaminated field soil increased the sensitivity of detection of spore balls in spiked soil samples four fold. The ability of the antiserum to discriminate contaminated field soil from an uncontaminated soil was much improved by using the gamma globulin fraction or cross-absorbed serum. Western blot analysis revealed that the antiserum detected a number of different proteins the most distinct of which had a molecular weight of slightly less than 6.5 KDa. A technique was developed to suppress autofluorescence of spore balls, allowing immunofluorescence studies to be carried out. Using this technique in conjunction with indirect FITC immunofluorescence discrete bright fluorescent spots were visualized using the specific serum. With the non-specific serum, only a very dull background fluorescence was evident.