LITERATURE REVIEW

Plants are usually immune to most microbial infections but sometimes they exhibit host-parasite relationship.The degree of relationship, however, varies from high compatibility to incompatibility. All plants irrespective of being susceptible or resistant have their own defence system but the spread and magnitude of response to their parasites differ. One of the most difficult and intriguing aspects in the study of biology is an understanding of the significant events of the interaction between plants and microorganism at the cellular and subcellular level. The success or failure of infection is determined by dynamic competition and the final out come is determined by the sum of favourable and unfavourable conditions for both pathogen and host cells. It is generally accepted that the cells recognize one another through pairs of complimentary structures on their surfaces. A structure on one cell carries encoded biological information that the structure on the other cell can decipher. Thus disease develops in individual plants by a series of sequential steps beginning with the arrival of the inoculum at the plant surface and ending with terminal stages of pathogenesis (Cowling and Horsfall; 1978). These stages from first to last are unique to each host and pathogen and also depends to a great extent on the prevailing environmental conditions. Considering the importance of serological relationships for host-pathogen interaction, a short selective review along these lines viz (a) Serological relatedness among fungal species; (b) Cross reactive antigens and (c) Detection of pathogen in host tissue has been presented below.

Serological Relatedness among fungal species

Many plant pathogenic fungal species are widely distributed and have broad host ranges and they are usually classified on the basis of morphological and cultural characteristics.However, taxonomic distinction between some species is often difficult due to similar morphology and disease symptoms are usually not diagnostic for species identification. Various techniques have been used to study taxonomic relationships of closely related fungi, including serological analysis (Krywienczyk and Dorworth, 1980 Hardham *et. al.* 1986). A possible, new approach for studying taxonomic relationships and for the identification of particular fungi involves the use of polyclonal and monoclonal antibodies.

Burrell *et al.* (1966), serologically studied three species of *Phytophthora,* by means of gel diffusion and immunofluorescence and found, that the nitrogen source, the amount

of inoculum and the age of mycelium greatly affected the antigenic efficacy of the preparations. The antigens were found to be localized in the young growing tips of the hyphae. Species-specific sera were obtained and proved to be useful and efficient for the identification of *P. cactorum, P. cinnamomi* and *P. erythroseptica.*

Serological differentiation in the genus *Ceratocystis* by using the serological techniques of agglutination, gel diffusion and immunofluorescence was reported by Amos and Burrell (1967). Eight species within the genus *Ceratocystis* were identified. All these serological techniques proved to be applicable in the identification of three fungi.They found that it was necessary to assess selectively cross-reacting antibodies from the sera to make them species specific. The immunofluorescence techniques proved to be most useful in differentiating among these species. Although all of the species were shown to have serological differences, no differences could be seen in the A and B compatibility types of *C. fagacearum.*

Immunodiffusion tests were used to study the taxonomic relations of six heterothallic species of *Phytophthora. P. cinnamomi* was serologically distinct, where as two serological groups were evident among the five closely related species. When tested with antiserum to *P. arecae, P. citrophthora,* and one isolate of *P. palmivora,* comprised one group, and *P. meadii, P. mexicana* and one isolate of *P. palmivora* the second group. The five species were serologically identical when tested with antisera to either *P. meadii* or *P. palmivora.* On the basis of their results Merz *et al.* (1969) suggested that five species might best be incorporated into one, *P. citrophthora.*

The immunological responses of *Verticillium alboatrum* and *V. nigrescens* pathogenic to cotton were compared. On basis of antigenic pattern *Verticillium* sp. were definitely differentiated from one another. Defoliating strain (Tg) of *V. alboatrum* was shown to differ antigenically from the non defoliating strain (SS4). It appeared to be more closely related serologically to the mildly virulent *V. nigrescens* isolates than was the defoliating T9 isolates (Wyllie and Devay, 1970).

A strain specific antiserum against the mature hyphae of *Fusarium culmorum* was prepared by Hornok and Jagicza (1973). Both the direct and indirect methods of the fluorescent antibody technique resulted in a specific yellow-green immunofluorescence, however, indirect staining was always more intense. They found that there was a significant

difference in the intensity of fluorescence between the mycelia of the homologous *F. culmorum* strain and those of the heterologous *F. graminearum* and *F. culmorum* strains. All the other *Fusarium* species tested showed no detectable fluorescence. The heterologous strains of *F. culmorum* and *F. graminearum* could not be distinguished. No significant difference was found between the 'culmorum' strains from different host plants. The serum specific of *F. culmorum* and *F. graminearum* is suitable to separate these species from other fungi.

Soluble proteins from the mycelia of 30 isolates of *Phytophthora cinnamomi* and of five isolates of *P. cactorum* when fractionated by disc electrophoresis yielded, 22 and 26 bands with different densities (Gill and Zentmyer, 1978). The two species differed markedly and each exhibited its distinct, characteristic protein pattern to identify them. They found that with one exception, there was little or no variation in the protein patterns within the isolates of *P. cinnamomi.* Also identical or nearly identical protein patterns of each species were obtained regardless of date of isolation, host, or geographic locality. No differences in protein patterns were seen between the mating types of *P. cinnamomi.*

Savage and Sall (1981) used Radioimmunosorbent assay, (RISA) for detection of *Botrytis cinerea* in homogenized samples. They showed that as little as 100mg of original fungal dry wt. can be detected. The assay was highly specific for *B. cinerea* although some showed 48% reactivity relative to *B. cinerea* and species of *Sclerotinia* and *Monilinia* showed 10-24% reactivity. All other fungi tested showed less than 0.1% reactivity. The usefulness of the assay for detection of the fungus within host tissue was demonstrated by the high correlation (r = 833) of the assay results with an estimation of rot weight from field infected lots. Artificially produced infection levels representing 0.1% infected tissue mixed with tissue homogenates were easily distinguished from background by the assay. They found, that the use of microtiter plates made of polyvinylchloride greatly increased the response of the assay so that its resolving power was improved.

The serological cross-reactivity of *Sporothrix schenckii* with various unrelated fungi was investigated by Ishizaki *et. al.*, (1981) using immunodiffusion tests. A rabbit anti-*S. schenckii* serum was obtained, which reacted with *Clasdosporium werneckii, C. carrionii, C. bantanum, Coccidioides immitis, Phialophera jeanselmei, P. gougerotii, P. dermatitidis, Fonsecaea pedrosoi, Aspergillus fumigatus, Histoplasma*

capsulatum and *Saccharomyces cerevisae* antigens. The serological determinants responsible for the cross-reactions were suggested by them to be D-galactosyl residues.

Iannelli *et al.* (1982) differentiated four formae speciales of *F. oxysporum* (*dianthi, melonis, pisi, lycopersici*) and three Physiological races (1, 2, 3) of *F. oxysporum* f. sp. *melonis* using specific antisera and the techniques of double diffusion and radio double diffusion in agar. Three isolates of *F. oxysporum* f. sp. *dianthi* race 2 and 10 of sp. *lycopersici* race 1 tested against the appropriate antisera reacted the same within each race.

Phytotoxic protein - lipo polysaccharide (PLP) complexes were isolated by Nachmias *et. al.*, (1982) from dialyzed culture fluids of a pathogenic strain of *Verticillium dahliae.* PLP complex which look phytotoxic activity were also isolated from a mutant non-pathogenic strain of the fungus and compared. A comparison profiles of wild type and mutant PLP eluting from an Agarose A-5 M column revealed quantitative differences between the 2 major protein peaks of each strain. The corresponding peaks of the 2 strains had similar molecular weights and in gel immunodiffusion were antigenically indistinguishable, but differences between them were seen in their chemical composition when analysed for protein, lipid and carbohydrate contents. Antiserum prepared against the components of peak 1 from the pathogenic strain reacted with an antigen in extracts of *Verticillium*- infected potato tissue which was apparently identical to a moiety produced by the pathogen in culture.

Immunological comparisons of teliospore surfaces of two Bunt fungi *Tilletia controversa* and *Tilletia caries* using polyclonal antisera and monoclonal antibodies indicated that both were very similar (Banowetz *et. al.*, 1984). Spectrophotometric analysis of surface extracts also indicated no extractable protein. None of seven fluorescein-labelled lectins bound these teliospores, even after the spores were treated with 8M urea to enhance exposure of potential lectin-binding sites. No quantitative distinction of the two species was found by any of the methods used in this study. However, 'double antibody sandwich enzyme linked immuno-assay DAS-ELISA) demonstrated quantitative differences in the numbers of certain monoclonal antibody binding sites of the two fungi.

Ala EL- Dein and EL-Kady (1985) used crossed immunoelectrophoresis (CIE), CIE with an intermediate gel and CIE with antibody absorption *in situ* techniques for comparison of the antigenic structures of *Botrytis cinerea, B. tulipae, B. paeoniae* and *B. allii* isolates. Antisera against antigens of these isolates, gene 24, 15, 20 and 15 precipitin peaks, respectively, when analysed in homologous reactions. CIE with an intermediate gel and CIE with antibody absorption *in situ* revealed that each isolate was serologically different from the other and had species-specific antigens. Eight antigens distinguished *Botrytis cinerea* from the other species of *Botrytis,* these were present only in the former species. *Botrytis allii* had less common antigens than the other species.

Immunofluorescence tests, involving 34 species of fungi were carried out by Chard *et. al.*, (1985) on an anti *Mycena galopus* serum raised against partially purified antigenic fraction. Cross-reacting fluorescence was produced primarily by *Mycena,* deutromycete and ascomycete species, non-*Mycena* basidiomycetes generally showing less fluorescence. Absorption of the antiserum with mycelium from cross-reacting fungi resulted in a reduction in fluorescence of cross reacting species, mostly to an acceptable control level.

Cross-reactivity between antisera produced against fimbrie of either *Ustilago violacea* (AV), or of *Rhodotorula rubra* (AR) and cell surface proteins of two ascomycete fungi, *Ascocalyx abietina* and *Ophiostoma ulmi* was revealed by means of dot-immunobinding and immunocytochemical methods. Following treatment with antiserum AR, the walls, septa, and plasma membrane of *A. abietina* and *O. ulmi* cells were appreciably labelled by gold particles of which labelling intensity was higher in plasma membrane. No significant labelling with this antiserum occurred over cells of *O. ulmi* indicating that they either lacked these antigens or that these were more easily removed during the fixation process (Benhamou *et al.* 1986).

Agglutination response of the conidia of eight *Fusarium* species to lectins having different sugar binding specification were examined by Cristinzio *et. al.*, (1988). Conidia of all the strain of *F. solani* and *F. oxysporum* were strongly agglutinated by concanavalin-A and *Helix Pomatia* agglutinin; while D-mannose (and D-glucose) and N-acetyl-D-galactosamine inhibited the agglutination induced by concanavalin A and *Helix Pomatia* agglutinin, respectively. They examined conidia of other species of *Fusarium (F. culmorum, F. sambucinum, F. graminearum, F. avenaceum, F. moniliforme* and *F. xylarioides*)

and found that they were not agglutinated by either lectin. No conidia from the *Fusarium* species were agglutinated by wheat germ agglutinin, potato lectin and *Ulex eropacus* agglutinin - I. These results have provided some insight into the chemical nature of the outermost layer of *Fusarium* conidia and this information may be of taxonomic significance.

Fuhrmann *et al.* (1989) obtained antisera from rabbit immunized with antigens of *Penicillium verrucosum* var. *verrucosum* and characterized by immunofluorescence as well as indirect enzyme-linked immunosorbent assay for their reactivity with 44 strains of moulds. Antigenically, *P verrucosum* var. *Verrucosum* (sub genus *Pencillium*) appears to be similar to strains belonging to subgenus *Furcatum*, but strongly different from *Penicillium frequentans* (sub genus *Aspergilloides*). Specific absorption of structures were noted on *Penicillium frequentans*, *Aspergillus versicolor*, and *Aspergillus fumigatus*. Immunological procedures may thus significantly contribute to refine the taxonomic classification of moulds.

Antiserum (anti-PfM) raised against mycelial suspensions of *Phytophthora fragariae* isolates reacted strongly with antigens from several *Phytophthora* species. Some cross-reactions with antigens from *Phythium* species were decreased by fractionating on an affinity column of Sepharose 4B bound to extracts of *Fragaria vesca* roots infected with *fragariae*. Mohan (1989), tested the affinity-purified anti-PfM retaining its high cross reactivity with the various *Phytophthora* species. He also detected infection of raspberry and strawberry roots by some *Phytophthora* species. This antiserum could, therefore, prove useful as a broad spectrum *Phytophthora* detecting antiserum. Anti-PfM could not be made specific for *P. fragariae* because it was raised against components shown to be antigenically similar in all *Phytophthora* species tested. However, immunoblotting with the affinity-purified anti-PfM produced distinct patterns for *P. fragariae*. *P. erythroseptica* and *P. cactorum* : three serotypes were identified for the latter species.

The potential of polyclonal antisera and monoclonal antibiodies to differentiate aggressive subgroups of *Ophiostoma ulmi* was explored by Dewey *et al.* (1989). Out of 33 cell lines that secreted monoclonal antibodies specific to *O.ulmi*, one third were non-specific, 11 were specific either to species or subspecies. Two cell lines differentiated mycelial antigens of the aggressive isolates of *O. ulmi* from those of the non aggressive

subgroup. Almost all the monoclonal antibodies and antiserum recognized antigen present in surface washings of cultures on solid medium, in cell free extracts of mycelial homogenates, in cell free culture fluids, and' in substances secreted overight by germinating spores.

Nameth *et. al.*, (1990) developed monoclonal antibody (LK50) against *Leptosphaeria korrae* (strain ATCC 56289) causal agent of necrotic ring spot disease of turfgrass. The antibody (MAb) was capable of detecting *L. korrae* in cultures and in the naturally infected blue grass samples. In cross reactivity tests using indirect ELISA, MAb reacted positively to all 24 isolates of *L. korrae*, MAb reacted negatively with 38 of 42 isolates of related and non related fungi and negatively to apparently healthy grass.

Guranowska and Wolko (1991) compared the extracts from *Fusarium oxysporum* (F.o.) and *Fusarium oxysporum* var *redolens* (F.o.r) isolates by means of electrophoresis and crossed immunoelectrophoresis. The polymorphism of fine isozyme systems allowed a distinction between *F. o* and *F.o.r.* isolates. The isozyme patterns of three other isozyme systems did not allow this distinction between *F.o.* and *F.o.r* to be made. Both fungi appeared almost identical serologically. Relative amounts of their corresponding proteins differed but the qualitative patterns of the proteins were nearly the same with the anti *F.o.r.* serum. Only one specific antigen was detected in the extracts from *F.o.r.* isolates Although the results obtained indicate a strong similarity between *F.o.* and *F.o.r.* they are not sufficient for an unequivocal statement that the fungi belong to the same species.

White *et al.* (1994) raised two monoclonal antibodies and three polyclonal antisera to cell wall / membrane fractions of *Pythium violae and Pythium sulcatum.* When they screened with a collection of 40 isolates of the genus *Pythium* including 20 species and the H-S group, they found extensive cross-reaction. However, when the binding of the antibodies was assessed in an enzyme-linked immunosorbent assay using cytoplasmic fraction antigens, the combined recognition patterns produced profiles unique to each species. On the basis of these profiles they used multivariate analysis methods to establish relationship between isolates.

Monoclonal antibodies (MAbs) based immunoflourescence test for detection of *Botrytis cinerea* on cut flowers were developed by Jesus and Arjen (1994) .Three selected MAbs recognized conidia of 43 isolates of *B. cinerea* from hosts. The percentage of conidia that fluoresced ranged from 50 to 100%. Intensity of fluorescence was related

more to the MAb than to the *Botrytis* isolate tested. MAbs showed no reaction with healthy flowers or to spores produced by other common airborne fungi and bacteria. Cross reaction with conidia of four other species of *Botrytis* occurred, but their fluorescence patterns differed from those of conidia of *B. cinerea.*

Ten monoclonal antibodies were selected from mice immunized with a highly purified elicitin secreted by *Phytophthora cryptogea* termed as cryptogein (Devergne *et. al.*, 1994). These antibodies could be classified into five groups according to their cross-reactivity to heterologous eliciting from other *Phytophthora* species, from strict specificity (reacting solely with cryptogein) to broad reactivity (reacting with all four eliciting under study). When examined on a real-time bio specific interaction analyser (BIA), these monoclonal antibodies were found to recognize at least three different epitopes on the cryptogein molecule. Their use in elicitin detection and quantification was optimized in several ELISA protocols. Using mixed monoclonal polyclonal antibody, indirect DAS-ELISA procedure detected as little as 20 pg of purified elicitin per well (100 µl). The four elicitins could be detected with the aid of one of couple of polyvalent reagents, while each one could be detected separately using appropriate monoclonal antibodies.

Cross Reactive Antigens

The serological relationship between plants and their fungal parasites have been repeatedly demonstrated over the years. There is evidence that host-parasite compatibility is related to their antigenic similarity. The presence of cross-reactive antigens (CRA) between plant hosts and their parasites and the concept that these antigens might be involved in determining the degree of compatibility in such interactions have been demonstrated by several authors. A number of reviews have been published previously in this area (Devay and Adler, 1976; Damien, 1964; Clark, 1981; Chakraborty, 1988; Purkayastha, 1989; Purkayastha *et. al.* 1991 and Purkayastha, 1994).

Various serological methods for the differentiation between resistant and susceptible varieties of cotton infected with *Fusarium oxysporum* and *Citrus* sp. with *Phytophthora citrophthora* have been described by Abd-EL-Rehim and Hashem (1970) and Abd EL Rehim *et. al.*, (1971a). Serological and immunoelectrophoretical investigation on water melon varieties, resistant and susceptible to *Fusarium semitectum* also revealed that the cultivars could be differentiated by the titre or the time after which reaction occurred between

antisera specific to the pathogens and seed globulin. It was noted that a, b globulin was present only in the resistant varieties. (Abd-EL-Rehim *et al.*, 1971b).

A common antigenic relationship between *zea mays* and *Ustilago maydis* was detected by Wimalajeewa and DeVay (1971). A pair of compatible haploid lines and two diploid solopathogenic lines of *U. maydis* were used in serological studies. *Avena sativa* var. 'Victory' and *Hordeum vulgare* var. California Mariout' were taken as resistant hosts. Certain antigens were found common between corn and *U. maydis*.

Charudattan and Devay (1972) compared preparations of antigens from four cotton varieties and isolates of *Fusarium* and *Verticillium* species for common antigens. At least antigenic substance was common among the varieties of cotton and isolates of *Fusarium oxysporum* f.sp. *vasinfectum*, *F. solani* f. sp. *phaseoli*, *Verticillium alboratrum* and *V. nigrescens*. Cotton varieties which were resistant or susceptible to Fusarium wilt as well as pathogenic and nonpathogenic isolates of *F. oxysporum* f. sp. *vasinfectum* shared the common antigen. The common antigen was not shared between *F. moniliforme* (nonpathogenic) and cotton. In gel-diffusion tests, five to eight precipitin bands were observed in the homologous reactions; of these, only one or two bands were common in hetrologous reactions between the fungal and cotton preparation. The common antigenic determinant shared by cotton and the fungal isolates does not appear related to the severity of wilt symptoms, but it may affect host-pathogen compatibility during the process of root infection.

Abbott (1973) determined the antigenic affinity among the saline soluble proteins of *Triticum aestivum* and *Avena sativa* and soil borne fungus *Ophiobolus graminis*. Single precipitin band in immunodiffusion test was formed when antisera of the wheat and oat roots were allowed to diffuse with the antigens of *O. graminis*.

The antigenic relationship between host and parasite in *Fusarium* wilt of cotton was also studied by Kalyanasundaram *et al.* (1978). Common antigen was shared by both avirulent and virulent isolates of *Fusarium oxysporum* f. sp. *vasinfectum* with disease resistant and susceptible line of cotton. In all cases, the fungal isolates invaded and parasitized cortical tissues of cotton roots, but only those fungal isolates that caused disease became established in the vascular system. Conidia of *Fusarium oxysporum* f. sp. *vasinfectum* contained an antigen that cross-reacted with antiserum to cotton root tissue antigens.

Charudattan and De Vay, (1981) isolated the cross-reactive antigen (CRA) from fungal conidia purified and partially characterized them. The CRA migrated as a single band in polyacrylamide or agar-gel electrophoresis, and sedimented as a single band during analytical ultracentrifugation. It was a protein-carbohydrate complex.

De Vay *et al.* (1981) also performed 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 epidermal cells and was present throughout the cortex tissue. Protoplasts prepared by them from cross-sections of young cotton roots also contained the CRA which was concentrated in the region of the plasmalemma. Treatment of the conidia and mycelia of F.o.v. with antiserum to cotton and using indirect staining the FITC indicated that the CRA was mainly present in hyphal tips and in patch-like areas on conidia.

Rabbit antisera were raised against the antigens of *Macrophomina phaseolina* (isolate MPI) and roots of soybean cultivars viz. Soymax and UPSM-19, susceptible and resistant respectively to charcoal rot disease. These antisera were used in immunodiffusion and immuno-electrophoretic tests for the presence of common antigens between isolates of *M. phaseolina* and soybean cultivars. Four antigenic substances were found common between the susceptible soybean cultivars and isolates of *M. phaseolina* but no common antigens were detected between resistant cultivars and the fungus. (Chakraborty and Purkayastha, 1983).

Using ammonium sulphate and ion-exchange chromatography fractionation Chard *et al.* (1985) partially purified the antigenic determinants specific for *Mycena galopus* and assessed the fractions by immunodiffusion and immunoelectrophoresis for the presence of the specific precipitation line.

Cross-reactive antigens were detected 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 Alba and De Vay (1985) using an Indirect enzyme linked immunosorbent (ELISA) technique.

Immunodiffusion, immuno-electrophoretic and crossed immunoelectrophoretic analysis of rice antigens using polyclonal antisera raised against *Acrocylindrium oryzae*

was done by Purkayastha and Ghoshal (1985). When the antigen preparation of *A. oryzae* was cross reacted with its own antiserum or against the antisera of four susceptible rice cultivars one precipitin band was detected. However, no precipitin band was detected when antiserum of the resistant cultivar was cross reacted with antigen preparations of three isolates 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 (1987) compared the antigenic preparations from two isolates of *Macrophomina phaseolina* causal agent of root rot of groundnut, four non pathogens of ground nut (viz. *Corticium sasakii, Colletotrichum lindemuthianum, C. corchsi*, and *Botrytis alii*), and five cultivars of groundnut using immunodiffusion, immunoelectrophoretic, and crossed immunoelectrophoretic techniques to detect cross-reactive antigens. 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.

Chakraborty and Purkayastha (1987) reported the changes in antigenic patterns after chemical induction of resistance in susceptible soybean cultivar (Soymax) to *Macrophomina phaseolina,* Sodium azide (100µg/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 (100µg/ ml).

Purkayastha and Banerjee (1990) used six antibiotics as foliar spray on a susceptible soybean cultivar ('soymax') to induce resistance against anthracnose. In addition, common antigenic relationship between seven soybean cultivars, their pathogens and non-pathogen were also studied using immunodiffusion, immunoelectrophoresis and indirect ELISA technique. Among the six antibiotics tested cloxacillin and penicillin induced maximum resistance against anthracnose. Cloxacillin (100 µg/ml) also altered the antigenic pattern of treated leaves. They detected cross

reactive antigens between susceptible soybean cultivars and the virulent strain of *Colletotrichum dematium* var. *truncata* but no cross reactive antigens were detected between soybean cultivars and avirulent pathogen (*C. dematium*) or non-pathogen (*C. corchori*).

Rabbit antisera were raised against three strains *Myrothecium roridum* (M-1, ITCC-1143, ITCC-1409), two susceptible cultivars (DS-74-24-2 and PK-327) and one resistant cultivar (UPSM-19) of soybean for analysis of cross reactive antigens shared between host and parasite. Results of immunodiffusion revealed that common antigens were present only between the virulent strain and susceptible host-cultivars. But no cross reactive antigen was detected in case of resistant cultivars (UPSM-19 and DS-73-16). Immunoelectrophoretic analysis showed that one common antigen was shared by susceptible hosts and the virulent strain (M-1). This was further confirmed by both crossed and rocket immuno-electrophoresis.(Ghosh and Purkayastha, 1990).

Serological relationship between *Sclerotium rolfsii* and groundnut cultivars were studied by Purkayastha and Pradhan (1994). Among three strains (266, 23, M) of *S. rolfsii,* 266 was most virulent and exhibited antigenic relationship with susceptible cultivars (Gangapuri, J-11 and AK-12-24) of ground nut. The strain 23 also exhibited common antigenic relationship with cv. AK-12-24. Resistant cvs. JL-24 and ICGS-26, however, showed no antigenic relationship with fungal strains. A systemic fungicide kitazin EC 48% (500µg/ml) altered the antigenic pattern of roots of a susceptible cv. AK-12-24 and also reduced disease markedly.

Antigens obtained from tea varieties, isolates of *Bipolaris carbonum* and non pathogens of tea *B. tetramera* and *B. setariae*, were compared by immunodiffusion, immunoelectrophoresis and ELISA to detect cross-reactive antigens (CRA) shared by the host and the parasite. CRA were found to be in the susceptible varieties (TV-9, 17 & 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 of (TV-18) and indirect

staining with FITC indicated the presence of CRA in the young growing hyphal tips and conidia. (Chakraborty and Saha, 1994).

Polycolonal antisera were also raised against mycelial suspensions of *P. theae* (isolate-pt-2), causal agent of grey blight disease and leaf antigens of Teen-Ali-17/1/54/ and CP-1 and immunological tests were performed in order to detect cross reactive antigens (CRA) shared by the host and parasite. CRA were found among the susceptible varieties and isolates of *P. theae* (pt-1, 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 staining of antibodies using FITC also indicated the presence of CRA in the epidermal cells and mesophyll tissues of tea leaves. CRA was evident in the young hyphal tips of the mycelia and on the setulae and appendages of the conidia of *P. theae* (Chakraborty *et. al,* 1995).

Among the tested varieties, TV-18 and Teen-Ali 17/1/54 were found highly susceptible, while CP-1 and TV-26 were resistant under identical conditions. Plant antigens were prepared from healthy leaves of five selected varieties and fungal antigens. Another serological experiment was performed by Chakraborty *et. al,* (1996) by raising polyclonal antisera against leaf antigens of tea varieties (TV-18, Teen-Ali 17/1/54 and CP-1) and mycelial antigens of *G.cingulata* (isolate GC-1) separately in white rabbits. CRA were found among the susceptible varieties and *G. cingulata* isolates. Such antigens were not detected between *G. cingulata* and resistant varieties of tea, non-pathogens and tea varieties as well as *G. cingulata* and non pathogens. In cross section of tea leaves (TV-18), the CRA was found to be concentrated in epidermal cells, mesophyll tissue and vascular elements.

Cross reactive antigens shared by *Fusarium oxysporum* and *Glycine max* were also detected using indirect ELISA and their cellular location in root tissues were detected using indirect immunofluorescence test by Chakraborty *et. al* (1997). For this, polyclonal antisera were raised against the mycelial suspension of *F. oxysporum* and root antigen of the susceptible soybean cultivar (UPSM-19). The immunoglobulin (IgG) fraction of those antisera were purified by ammonium sulfate precipitation and DEAE- sephadex column chromatography. Antigens of susceptible cultivars showed higher absorbance values than resistant cultivars when tested against the purified anti *F. oxysporum* antiserum. Indirect

Fluorescence tests using FITC indicated that in cross-sections of roots of susceptible cultivar (UPSM-19) CRA were concentrated around xylem elements, endodermis and epidermal cells while in resistant varieties fluorescence was concentrated around epidermal cells.

Detection of Pathogen in Host tissue

The development of serological techniques have produced a number of highly sensitive methods for detecting microorganisms in diseased plants. These techniques are highly specific and has the ability to detect even very small amount of pathogen in the host tissues. A number of recent reviews have been published by workers in this direction (Hansen and Wick, 1993 and Werres and Steffens, 1994).

Species of *Aspergillus* and *Penicillium* was found to be predominant fungi from 5 samples of stored grains when isolated in culture. By using an immunofluorescence method Warnock (1973) confirmed the presence of these fungi in most grains, but the amounts of mycelium involved were small. *Alternaria* sp. were not isolated from 3 of the samples but small amounts of *Alternaria* mycelium were detected in grains of all the samples studies.

Casper *et. al,* (1979) used the technique of enzyme-linked immunosorbent assay (ELISA) to estimate *Verticillium lecanii* at different stages of infection in leaves of wheat heavily infected with yellow rust (*Puccinia striiformis*).

Duncan (1980) detected the red stele pathogen, *Phytophthora fragariae* by a "root-tip bait" test in certified commercial stock of strawberry plants, supposedly free of disease. Susceptible bait plants were grown in a mixture of compost and root tips cut from runner plants. When root tip samples were prepared from a combination of 1:99 infected / un-infected runner plants, the test gave positive results in all 10 trials.

Marshall and Patridge (1981), raised antibodies to the ribosomes of *Fusarium moniliforme* and corn (*Zea mays L.*) and showed them to be specific by the Ouchterlony double-diffusion test and sucrose-density gradient analysis of the antigen-antibody reaction.

Antiserum to homogenates of washed *Epicholoe typhina* mycelium grown in liquid medium was prepared and used in an enzyme-linked immunosorbent assay (ELISA) to

detect antigens of the fungus in tall fescue tissue samples. With ELISA as little as 100mg of freeze dried *E. typhina* mycelium per milliliter, was detected. Of 14 fungal genera tested, including *Acremonium, Claviceps, Helminthosporium, Pythium, Rhizoctonia* and *Sclerotium*, all showed reactivities less than 0.1% that of *E. typhina* (Johnson *et. al,* 1982).

Antisera were made to both a whole cell and cell wall preparation of *Eutypa armeniaceae*. Gendloff *et.al.* (1983), tested Rhodamine isothiocyanate (RITC) conjugated antisera for reactivity with various fungi on glass slides. They found that, both the 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. armeniaceae* 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 serum then stained wtih RITC-labelled goat anti-rabbit gamma globulin. Both procedure specifically stained hyphae in wood sections. Hyphae stained indirectly in woody vine sections showed a much higher 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.

Antisera to *Phoma exigua* var. *foveata* and var. *exigua* were prepared by Aguelon and Dunez (1984) by injecting rabbits with protein solutions from mycelium. Immunoenzymatic techniques (DAC and DAS ELISA) were used to test for the fungus 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 *et.al.* (1984), provided an interesting practical application of the fluorescent antibody technique for the detection of *Ganoderma lucidum* int the roots of betel nut.

In liquid culture, *Phaseolus schweinitzii*, causal agent of root rot of conifers, secretes a number of species specific and stain specific polypeptides which are detectable by SDS-

PAGE and isoelectric focussing. Dewey *et.al,* (1984) used indirect immunofluorescence microscopy to detect the binding of species-specific antisera to extracellular macromolecules secreted by the fungus, to the cell surface of basidiospores and chlamydospores, and to the cell surface and cross walls of mycelia. Common antigenic determinants were present in extracellular culture filtrate material and walls of mycelia, chlamydospores, and basidiospores. They demonstrated the presence of mycelium and on occassions chlamydospores in naturally and artificially infested soil samples by indirect immunofluorescence using antisera to culture filtrate molecules.This makes possible identification of the kind of propagule most likely to be the source of field isolates of the organisms.

Walcz *et al.* (1985) described ELISA techniques for the detection of *Sclerotinia sclerotiorum* from both artifically and naturally infected host plants. Such technique showed good specificity and sensitivity in detecting *'Sclerotinia'* antigen at a concentration as low as 10ng/ml.

Gerik *et.al.* (1987) detected hyphae of *Verticillium dahliae* in cotton root tissue with indirect ELISA. 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* were readily observed on and in root cortex of the host using a dissecting microscope. This technique allowed for rapid determination of the presence and location of *V. dahliae* in the root systems and may be applicable to other fungal species for which a specific antiserum can be produced.

The surface deposition and accumulation of cerato-ulmin by the aggressive isolates of *Ophiostoma ulmi* were demonstrated by Svircev *et.al.* (1988). The protein A-gold complex was present on the surface of the vegetative hyphae, synnemata, synnematal spores, perithecia and ostiolar hairs of the aggressive isolates of *O. ulmi* containing the toxin cerato-ulmi (CU). They found that the protein A gold label was either evenly distributed on the fungal surface or in the form of large surface aggregates. The non-aggressive isolate (Q412) of *O.ulmi* had a low concentration of protein A-gold label on its fungal structures. When the specific CU antiserum was replaced by preimmune serum, a lack of the protein A-gold label was evident on all fungal structures of the aggressive isolate of *O. ulmi.*

Colonization of field-grown cotton roots by *Verticillium dahliae* was studied by Gerik and Huisman (1988) by using a specific immunoenzymatic staining technique. They found that the colony densities of *V. dahliae* increased with distance from the root tip with the maximal density occuring more than 1 cm from the root apex. The mean colony length of *V. dahliae* was 7.3 mm, 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 appearance was consistent with growth of hyphae from the root surface toward the stele.

Amouzou-Alladaye *et. al.* (1988) obtained rabbit antisera against mycelial protein extracts of one strain of *Phytophthora fragariae* which had a dilution end point of 1/64 in double diffusion and 1/512,000 in indirect ELISA. They found that this serum could detect 11 different strains of *P. fragariae* and the pathogen in naturally infected or inoculated roots. Although the sensitivities of direct double antibody sandwich (DAS) and indirect ELISA were comparable, the 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 DAS-ELISA several days before oospores were found and before symptoms developed.

Unger and Wolf (1988) have presented an indirect ELISA for quantitative detection of *Pseudocercosporella herpotrichoides* infections in wheat. All tested isolates reacted on a high level. No cross reactions occur 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. They examined 24 stem-base samples from the field which showed that the values assessed by ELISA correlate well, also with the disease indices of naturally infected plant material.

Mohan, (1988) could also detect fungal antigens in extracts of 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 Gaunlet, which is resistant to Pf 1, 2 and 3 but susceptible to Pf 10 and 11, reflected this differential response in ELISA : He found that 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 virulent isolates. The recently introduced cultivars Hapil, Ostara and Providence were found to be susceptible to all isolates in this study : the corresponding root extracts were also positive in ELISA. The antiserum also detected *P. cactorum* infections. Nevertheless, the ELISA test described should prove valuable in screening certified strawberry stocks.

Sengupta *et. al.* (1989) studied the outer membrane proteins (OMPs) of *Vibrio cholerae* strains of 01 and non 01 serovars. They found marked similarity in the OMP profiles of different *V. cholerae* 01 strains but the OMP profile of a non 01 strain was somewhat different. Antigenic relatedness between the OMPs of different *V. cholerae* strains was established by enzyme-linked immunosorbent assay (ELISA). Immunoblotting experiments were demonstrated by them which showed at least two OMPs of 36 and 25-26 Kda were immunogenic and common to strains of 01 and non 01 serovars.Antiserum raised against the outer membrane of a *V. cholerae* strain, and rendered specific for its OMP by absorption with lipopolysaccharide, inhibited in vitro the intestinal adhesion of the homologous and heterologous strains of *V. cholerae* irrespective of their biotype, serotype and serovar. Furthermore, antiserum to OMPs induced passive protection against *Vibrio* challenge in rabbit ideal loop experiments. These results suggest that the OMPs may be useful in immunoprophylaxis against cholera.Antiserum was raised against pooled mycelial suspensions from five isolates (designated Pf 1, Pf2, Pf3, Pf 10 and Pf 11) representing five physiologic races of *Phytophthora fragariae*. In enzyme linked immunosorbent assay (ELISA), this antiserum detected homologous soluble antigens at protein concentrations as low as 2 ng/ml.

Competitive types of two novel enzyme linked immunosorbent assays (ELISA) for *Fusarium* species were developed by Kitagawa *et. al.* (1989) Antiserum against a strain (F504) of *F. oxysporum* was elicited in rabbits, and a highly specific, sensitive and acurate ELISA for the homologous strain was developed by using the antiserum with

β-D-galactosidase labelled anti-rabbit IgG as the secondary antibody and cell fragments of the strain attached to Amino Dylark balls as the solid-phase antigen. All other microorganisms tested, including nine other strains of *Fusarium,* showed little cross-reactivity. When cell fragments of *F. oxysporum* (F501) attached to the balls were used as a solid-phase antigen in a heterologous competitive ELISA, the modified system was a general assay for 10 strains of four *Fusarium* species with a high sensitivity. The specificity of the heterologous competitive ELISA was shown to be limited to *Fusarium* species. The reason for developing the improved ELISA is also presented by them.

Lange *et.al.* (1989), raised an antiserum against *Plasmodiophora brassicae*, the causal agent of club root of cabbage. They used a semipurified suspension of spores of *P. brassicae* as antigen, obtained by filteration and Percoll gradient centrifugation of infected roots. Crude root suspension samples were bound to a nitrocellulose membrane and tested by a dot immuno binding assay. The individual steps of the serological procedure were examined with a scanning electron microscope. The surface of the resting spores of *P. brassicae*, race 7, appeared smooth, while the dot immuno binding proccessed spores had a heavy, irregular coating, which was demonstrated to originate from incubation in the primary antiserum. Normal serum did not give rise to a coating on the resting spores. The antiserum *P. brassicae* did not react with surface antigens of resting spores *Polymyxa.* Further no cross reaction with other common root pathogen such as *Pythium ultimum, Rhizoctonia solani* and *Fusarium oxysporum* was observed. With antiserum prepared against spore suspension antigenic determinants the dot immuno binding technique can be used as a routine test for detection of infection of *P. brassicae* in host plant and in bait plants (used as indicators of soil infestation). The sensitivity obtained was within the range permissible for a routine test.

Immunofluorescent antibody is an useful method for detecting *Phytophthora.* But the autofluorescence and the nonspecific staining of soil particles interfere with the detection of the fungi in soil . Watabe (1990) reported that the pretreatment of the samples with gelatin rhodamine conjugate, prevented the samples from the autofluorescence and the non-specific staining and therefore permitted the immunofluorescent antibody staining in soil. He detected stained *Phytophthora* easily on the yellow-orange background.

Phelps *et al.* (1990), developed an enzyme-linked immunosorbent assay for isomarticin, a naphthazarin toxin produced by *Fusarium solani.* They used a carbodiimide

procedure to couple the hapten isomarticin to bovine serum albumin for the immunogen and to alkaline phosphatase for the enzyme linked tracer. The resulting assay had a detection limit of 2 ng/ml for isomarticin. Other naphthazarin toxins were detectable at less than 10 ng per well in ELISA plates. The assay was specific for naphthazarins. The cross-reactivity for a number of phenolic compounds including the closely related naphthoquinones, was three orders of magnitude less sensitive.

Hardham *et al.* (1990) studied, Glycoconjugates on the surface of zoospores and cysts of the pathogenic fungus *Phytophthora cinnamomi,* using fluorescence isothiocynate labelled lectins for fluorescence microscopy and flow cytrometry, ferritin and gold-labelled lectins for ultra structural analysis. They found, that, of the five lectins used, only concanavalin A (Con A) binds to the surface of the zoospores, including the flagella and water expulsion vacuole. This suggests that of accessible saccharides, glucosyl or mannosyl residues predominate on the outer surface of the zoospore plasma membrane. Early in encystment, a system of flat disc like cisternae, which underline the zoospore plasma membrane, vesiculate. These and other small peripheral vesicles quickly disappear. After induction of encystment, Con A is no longer localised close to the plasma membrane but binds to material loosely associated with the cell surface. Quantitative measurements by flow cytometry indicate that the Con-A-binding material is gradually lost from the cell surface. The cyst wall is weakly labelled, but the site of germ tube emergence stains intensely. During the first 2 min after induction of encystment, material that binds soyabean agglutinin. *Helix pommatia* agglutinin, and peanut agglutinin appears on the surface of the fungal cells. The distribution of this material, rich in galactosyl or N-acetyl-D-galactosaminosyl residues, is initially patchy, but by 5 min the material evenly coats most of the cell surface, labelling of zoospores in which intracellular sites are accessible indicates that the soyabean agglutinin binding material is stored in vesicles that lie beneath the plasma membrane. Quantitation of soyabean agglutinin labelling shows that maximum binding occurs 2-3 min after the induction of encystment.

An immunocytochemical stain was developed by Young *et al.* (1990) to detect the basidiomycete *Athelia bombacina* in apple leaf litter. The polyclonal antibodies for *A. bombacina* were sufficiently specific and only hyphae of this fungus were detected in immunocytochemically treated sections of dead leaves inoculated with *A. bombacina.* Apple leaves naturally infected with *Venturia inaequalis* were inoculated with *A.*

bombacina incubated outside from November 1986 to May 1987, and sampled monthly. They found from the sections stained immunocytochemically, that *A. bombacina* grew endophytically and epiphytically. The antagonist prevented either growth of hypae of *V. inaequalis* into the interior leaves, nor initiation of pseudothecia. There was no particular spatial association between hyphae of the two fungi, nor any sign of direct parasitism of hyphae or pseudothecia of *V. inaequalis*. Pseudothecia in leaves with the antagonist did not mature further that the stage of producing pseudoparaphyses, reaching an average of 84 µm in length. Pseudothecia in leaves without *A. bombacina* developed asci normally and were 108 µm long by 1 May. These data were confirmed by result from an abbreviated sampling scheme of Mc Intosh apple leaves during 1987-1988 and by observations of pseudothecial inhibition in crab apple leaves following delayed application of the antagonist.

Harrison *et al.* (1990) raised polyclonal antiserum, in rabbit, immunized with a mycelium extract of *Phytophthora infestans*, and reacted in an enzyme-linked immunosorbent assay (ELISA) with mycelial extracts of two *Phytophthora* species but not with those of 10 other micro-organisms found on potato. *P. infestans* mycelium in leaf tissue was readily detected by ELISA using either the plate-trapped antigen of F. (ab)$_2$ 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.

Daniel and Nilssan (1991) raised polyclonal antiserum against mycelial extracts from the degrading soft rot fungus *Phialophora mutabilis*.In enzyme-linked immuno sorbent assays (ELISA), the antiserum reacted strongly with its homologous antigen and cross reacted strongly to moderately with six other soft rot *Phialophora* species. With the exception of *Ceratocystis*, the serum reacted weakly or not at all with 11 other mold, blue, and rot fungi occuring frequently in or on wood. They found that the antiserum cross-reacted strongly with antigens in extracellular filtrates from *mutabilis* cultures that contained about 40 ng/ml of protein. Ultra structural and immunocytochemical studies on wood degraded by *P. mutabilis* showed specific localization of the antibody on the fungal cell wall and certain intracellular structures. Extracellular labelling within soft rot cavities and sites of erosion decay of wood also were noted. The antiserum was assessed by ELISA for detecting the presence of the fungus and soft rot in untreated and preservative treated wood blocks of pine and birch degraded for periods of 1-12 months. *P. mutabilis* was

detected in samples from all wood blocks degraded to low or high weight loss. Highest ELISA reading were recorded by Daniel *et. al.* (1991) for wood blocks with highest substrate losses and vice versa.

Sundaram *et. al.* (1991) raised polyclonal antisera against purified mycelial proteins from *Verticillium dahliae*, the predominant fungus species in the potato early dying complex. The antisera was tested against crude mycelial preparations of *Verticillium* sp. used indirect enzyme linked immunosorbent assay (ELISA) reacted positively with 11 of 12 *V. dahliae* isolates from potato, cotton and soil but negatively with one isolate from tomato. They found, that the antisera did not react with mycelial proteins from *Fusarium* sp., from potato and cotton, with a *Colletotrichum* sp. from potato, or with one isolate of *Rhizoctonia solani* from sugarbeet.Double antibody sandwich ELISA using polyclonal antisera, detected *V. dahliae* and *V. albo-atrum* in infected roots and stems of potato.

Shane (1991) compared methods for sampling turfgrass tissue for their effectiveness in monitoring Pythium blight epidemics with enzyme linked immunosorbent assay (ELISA). He took sample areas which consisted of marked strips golf-course fairways and trees with bent grass and annual blue grass, naturally infested by *Pythium aphanidermatum*. (1) Sample consisted of whole plants picked by hand and assayed as whole plants, (2) whole plants sectioned into lower, middle and upper strata components and (3) leaf clippings collected with a reel mower set at a 1.2 cm cutting height, ELISA readings for mowed samples generally matched those for whole-plucked samples (r^2 values ranging from 0.457 to 0.601). He found fluctuations in detectable *Pythium* antigen more pronounced on the upper most 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, increases in ELISA readings for *Pythium* coincided with, but did not generally precede the on set of blight symptoms with a 2 to 3 day sampling interval Antibody aided detection is useful for verification of diagnosis and determination of general *Pythium* population fluctuations, but current methodology is not satisfactory for advanced detection of blight epidemics.

Ricker *et. al.* (1991) designed experiments to produce water-soluble antigens by *Botrytis cinerea* which were detected in spiked and naturally infected grape juice by using

an enzyme immunoassay with an indirect fromat of antibody horse-radish-peroxidase conjugates bound to polyclonal rabbit antibodies directed against *B. cinerea* (anti-BcIgG). Protein A purified gamma globulin from an early bled antiserum which reacted primarily with low molecular weight compounds present only in extracts of *B. cinerea*, was used to specifically detect *B. cinerea*, and quantity levels of infection in juice from infected grape berries. They used, late-bled, higher titer antiserum which cross-reacted with proteins and carbohydrates present in extracts from species of *Botrytis, Aspergillus, Penicillium* and *Uncinula*, to quantify levels of rot caused by the presence of multiple fungi. Minimum detectable levels of infection based on mixtures of clean and infected juice, were 0.25-0.5% with 803-7 IgG and 0.02% with 803-19 IgG by using antigen from *Aspergillus niger* coupled to Sepharose beads improved specificity of anti-BcIgG to *B. cinerea*, but decreased detection sensitivity to approximately 0.5% infection. Cross-reactivity of all anti Bc IgG collections was consistent low with juice extracted from uninfected grape berries. In contrast cross-reactivity of anti BcIgG with water soluble antigens extracted from sterile and reproductive structures of several fungi was found to be, negligible in early bled antiserum and increased in subsequent collections. The increase, in cross-reactivity in late-bled antisera correspond with an increase in the overall serum titers for anti BcIgG to antigens from *B. cinerea*. Nonspecific binding of 803-19 IgG was high with extracts from *A. niger* and an unidentified species of *Penicillium*, suggesting numerous epitopes common to antigens from these fungi.

Nemee *et. al*(1991) detected Naphthazarin toxins of *Fusarium solani* by competitive ELISA analysis in xylem fluid of roots rotted by *F. solani* and symptomless scaffold roots and branches of healthy appearing and diseased citrus trees in ridge and flatwoods Florida groves. Studies concentrated on blight, a wilt disease with an undefined cause and etiology, to determine if *F. solani* is a casual factor of the disease. They found that healthy appearing roots of trees with blight symptoms in six groves contained upto 11.4 times more toxin than roots of healthy trees in the same groves. In blight diseased trees from these groves, median toxin values per root and the percentage of roots positive for toxin were higher than for healthy trees. In two groves, one containing tristeza-diseased trees and the other foot rot diseased trees, toxin concentrations were greater in diseased compared with healthy trees only in the foot rot site. Toxin concentrations were not different healthy-appearing roots of healthy tangerine and sweet orange trees on *Citrus liman* (Milan) in adjacent groves in a

burrowing nematode site. Significantly more toxin was present in branches of blight than in healthy trees in two of three groves. In fibrous roots infected by *F. solani*, immunocytochemical localization of naphthazarins was present in fungal cell walls and associated electron dense substances on the outer surface of the hyphae. In the fungal cytoplasm, the toxin was localized in non membrane-bound electron-lucent areas. The presence of napthazarin toxins in blight diseased trees as well as these with other diseases suggests the non specificity of *F. solani* pathogenic activity on various root stocks. Therefore, in situ toxin concentrations high enough to trigger pathogenic effects in susceptible root stocks may be required to cause blight.

Gwinn *et. al.* (1991) determined the distribution of the endophytic fungus, *Acremonium coenophialum* within the tissues of infected tall fescue (*Festuca arundinaceae*) with tissue print-immunoblot (TPIB), however, the accuracy of this method in the determination of endophyte infestation levels of pastures and seed lots has not been evaluated. They have compared TPIB to other methods used for endophyte detection. They further tested seeds of known endophyte infestation levels with TPIB. Percentage endophyte infestation did not differ significantly from the expected values. Also, no significant differences were found between results of protein A-sandwich ELISA and TPIB tests when both were used to estimate endophyte infestation levels in pastures. Therefore, accuracy of TPIB is comparable to other endophyte detection techniques and can be used for routine detection of endophyte in tall fescue tissues.

Two commercial serological assay kits were composed by Benson (1991) to a culture plate method for detection of *Phytophthora cinnamomi* in root samples from inoculated azaleas. Both the multi well E. Kit and the rapid assay F. Kit detected *P. cinnamomi* on azalea roots begining 1 wk after inoculation. Agreement between immunoassay kits and culture plate results for detection of *P. cinnamomi* was most consistent begining 3-5 wk after inoculation. He found, root symptoms, but not foliar symptoms, of *phytophthora* root rot, 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 symptoms severity and immunoassay results. Although colour reactions in the rapid assay detectors became increasingly darker after completion of the test, results after 5 min were as reliable as those after 60 min since readings for uninoculated controls used to determine test

thresholds also increased with time. The multi well 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 at two nurseries had positive ELISA values that were un confirmed by culture plate. The rapid assay kit detected *P. cinnamomi*, was easy to use, and gave results in a short time.

Pscheidt *et. al,* (1992), tested the sensitivity of a *Phytophthora* specific immuno assay kit on 17 species of *Phytophthora* collected throughout the world, including 18 isolates each of *P. cinnamomi* and *P. cactorum.* Kits were also used in the diagnosis of plant specimens with symptoms characteristic of *Phytophthora* infection, which are sent to Oregon State University's Plant Disease Clinic. All *Phytophthora* isolates tested produced a positive reaction with the immuno assay kit. The lowest absorbencies relative to other species were obtained from *P. cinnamomi* and *P. megasperma* (Originally isolated from Cherry). Variation in absorbance was high among isolates of infection produced a positive reaction with the immunoassay, as did pure cultures of the immunoassay, as did pure cultures of *Phytophthora sp.* isolated from the samples. Cross reactions occurred with several *Pythium* sp. isolated from clinic samples and with several specimens infected with *Pernospora* sp. Other samples without typical *Phytophthora* symptoms, but associated with other pathogens did not produce a positive reaction with the immunoassay. Cross-reactivity with some *Pythium* sp. made interpretation difficult, but when kit results were combined with field histories and symptomology, the immunoassays proved to be a useful tool in clinical diagnosis.

Takenaka (1992), utilized ribosomal protein differences among snow mold fungi and wheat, to detect the causal fungi in infected wheat leaves by indirect enzyme linked immunosorbent assay (ELISA) and Western blot analysis. He raised polyclonal antiserums against the ribosoms of *Pythium paddicum, P. iwayami, Typhula incarnata* and *Microdochium nivale* and tested for sensitivity and specificity with ribosomes of these four pathogens, *Typhula ishikariensis*, and wheat. Using polyclonal antiserums, ribosomes of *P. paddicum* and *P. iwayamai* were serologically identical, the ribosomes of *T. incarnata* and *T. ishikariensis* had partial common antigenic determinants but there were apparent serological differences among *Pythium* sp. *Typhula* sp, *M. nivale*, and wheat. With indirect ELISA, ribosomes of *Pythium* sp. were detectable at a concentration of 69 ng/ml and ribosomes of *T. incarnata* and *M. nivale* at a concentration of 210 ng/ml. The

ribosomes of each target pathogen were detected from completely rotted wheat leaf homogenates diluted upto 1 : 1000 or 1 : 10,000. The indirect ELISA could not differentiate *P. paddicum* from *P. iwayamai* infected plants or *T. incarnata* from *T. ishikariensis* infected plants but could detect and differentiate snow mold fungi at the genus level in wheat plant. Western blot analysis with these antiserum also could not differentiate *P. paddicum* from *P. iwayamai* but could differentiate wheat leaves infected with *T. incarnata* from those infected with *T. ishikariensis*. These immunological methods with antiribosome serums could be useful to evaluate wheat plants for infection by *pythium* sp., *T. incarnata, T. ishikariensis* and *M. nivale*.

Conventional methods indicated that *Pythium violae* was most commonly isolated from carrot cavity spot samples from 14 UK sites. For one site the most frequently isolated species was *Pythium sulcatum.* Lyons *et. al.* (1992) designed methods where similar isolation work were compared with the assay of cavity spot lesions using polyclonal antibodies, raised to *P. violae* or *P. sulcatum* in competition ELISA. Where lesions were artificially induced the test confirmed which pathogen was causal. When 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 isolations were negative.

Polyclonal antibodies (PAbs) were produced in New Zealand white rabbits with culture filtrate and mycelial extract immunogen preparations from soyabean (*Glycine max*) fungal pathogen *Phomopsis longicolla.* The PAb's were purified to the immunoglobin fraction by Brill. *et.al.* (1993) and tested in indirect enzyme-linked immunosorbent assay (ELISA) and in double antibody sandwich-ELISA (DAS-ELISA). The PAb's 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. They performed DAS-ELISA which proved to be more specific and 100-fold more sensitive in detecting members of the complex than that of indirect ELISA. Immunization of one rabbit with culture filtrate over an extended time resulted in maximum anti *P. longicolla* activity after three immunizations, and the activity became constant against most members of the

complex at the same time. Reactivity to some cultures of *P. longicolla* was undetectable following the fourth and fifth immunizations, where as reactivity to all of the other cultures of the complex remained high.

Polyclonal antiserum, was elicited against a strain of *Fusarium oxysporum* f.sp. *narcissi* (GCRI 80/26) and a specific and sensitive enzyme-linked immunosorbent assay developed by Christine (1993). Antiserum raised to cell wall fractions gave better recognition than that to cytoplasmic fractions. Recognition was equally good in artificially and naturally infected bulbs. He showed that there was little cross-reactivity in bulb tissue 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 cross-reactivity was observed with two other *Fusarium* sp. Four *Fusarium* sp. 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. Christine, observed a direct correlation between positive results in the ELISA and recovery of the pathogen on selective medium.

Beckman *et. al.* (1994) developed a polyclonal, enzyme-linked immunosorbent assay (ELISA) of *Phytophthora infestans* for use in the determination of fungal biomass during early stages of infection of tuber discs of *Solanum tuberosum*. They optimized the dilution of sample extracts and dilution of primary anti - *P. infestans* antiserum, and the quantification of the biomass of *P. infestans* in zoospore inoculated tuber discs could be achieved by 8-18h after inoculation. Differences in growth between avirulent and virulent isolates of *P. infestans* on the resistant potato cv. Kennebee were quantified by 32-48h after inoculation. Together with a comparison of the growth of the same isolates on the susceptible cv. King Edward, these results comprised on ELISA of the Quadratic check. On the resistant host, the growth of the avirulent isolate was essentially arrested by 16h after inoculation, whereas that of the virulent isolate continued throughout the time course. They found that on the susceptible host, however, the avirulent isolate appeared more aggressive than the virulent isolate. These results demonstrated that the ELISA, which is often simpler to perform than other procedures for estimating fungal growth, may be used to complement biochemical studies of rapidly induced plant defence responses.

Jamaux *et al* (1994) developed a serological test which allowed the early detection of infection of young petals by . *Sclerotiorum*, an important pathogen of rapeseed. Two steps were required to obtain an antiserum sufficiently specific for *S. sclerotiorum*. they raised first generation of polyclonal antiserum by using soluble mycelial extracts of *S. sclerotiorum* in double antibody sandwich enzyme - linked immunosorbent assay (DAS-ELISA) and allowed the screening of cross-reacting fungal species such as *Botrytis cinerea* a pathogen commonly present on rape seed petals. Using a polyclonal anti *B. cinerea* serum enabled the absorption, by serial cycles, of *S. sclerotiorum* antigens common to *B. cinerea*. Residual antigens were then used as immunogens for the production of two seemed generation antisera (S1 & S2) which were then tested by DAS-ELISA.

Detection of field isolates of *Thielaviopis basicola* the causal agent of black root not of cotton were taken (*Gossypium hirsutum*) and grown in Czapek-Dox broth ammended with dialyzed carrot extract. Soluble protein extracts of chlamydospores and mycelium were used to raise polyclonal mouse ascites antibodies. The immunoglobulin G antibody fraction was purified and biotin-labeled to devise a fungal capture sandwich enzyme-linked immunosorbent assay (ELISA). ELISA detected both brown and grey cultural types of *T. basicola* and had negligible cross-reactivity with other soil borne fungi commonly found in the San Joa quin Valley of California cotton field soils. The minimum detection limit of ELISA was between 1 and 20ng. of *T. basicola* protein depending on the assay *T. basicola* could be detected in cotton roots 2 days after inoculation. At this time, initial symptoms were apparent. The antibody also used to observe *T. basicola* on cotton roots with immunofluorescence microscopy (Holtz *et al.* 1994).

Chakraborty *et al.* (1996) raised polyclonal antiserum against the mycelial extract of *Pestalotiopsis theae* and immunoglobulin fractions were purified by ammonium sulfate fractionation and chromatography on DEAE sephadex. In enzyme-linked immunosorbent assay, antiserum dilutioin upto 1:16000 detected homologous antigen at a 5mg/L concentration, and at 1:125 antiserum dilution fungal antigens could be detected at a concentration as low as 24 µg/L. In fifteen varieties of tea tested, originating from Darjeeling, UPASI and Tocklai breeding stations, absorbance values of infected leaf extracts were significantly higher than those of healthy extracts at a concentration of 40mg/L 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, the pathogen could be detected in inoculated

leaf extracts upto antigen concentration of 2mg/L. The antiserum reacted with two other isolates of *P. theae* tested but not with the antigens from mycelial extracts of *Glomerella cingulata* and *Corticium invisum* or with extracts of tea leaves inoculated with these pathogens. The results demonstrate that ELISA can be used for early detection of *P. theae* in leaf tissues even at a very low level of infection.

Enzyme linked immunosorbent assay using antiserum raised against *Colletotrichum falcatum* was performed in order to pathogen well before the symptom development. When different tissues in the infected sugarcane plants were screened for antigen titre, root eyes and bud in the nodal region and internodal samples from pith and white spot gave higher values showing more pathogen colonization. When 20 different sugarcane varieties were subjected to ELISA test after pathogen inoculation, it showed an clear variation in disease resistance among them as in field testing. (Viswanathan *et al.* 2000).

Immunological detection of *Sphaerostilbe repens*, *Trichoderma viride* and *Trichoderma harzianum* using DAC-ELISA. formats have been demonstrated by Chakraborty *et al.* (2000), in order to develop strategies for management of violet root rot of tea. Polyclonal antibodies (PAb) against mycelial and cell wall antigens of *Ustulina zonata* causal agent of charcoal stump rot of tea were also raised and IgG were purified using DEAE-cellulose chromatography. Effectiveness of antigen preparations for raising PAbs were done using DAC-ELISA. Two different ELISA formats such as direct antigen coated (DAC), and double antibody sandwich (DAS) were tested to detect the pathogen in soil and artificially inoculated tea root tissues (Chakraborty *et. al.* 2001a). Polyclonal antibody (PAb) was raised against immunogen preparations from mycelial extract of *F. lamaoensis*, causal agent of brown root rot disease of tea. Optimization of PAbs were done using indirect enzyme linked immunoassay (ELISA). Increased activity of PAbs against *F. lamaoensis* could be noticed from second bleedings, which continued upto fourth bleeding. DAC ELISA formats were developed to detect the pathogen in infested soil. (Chakraborty *et. al.* 2001b).