Literature Review

It has long been known that most plant pathogens possess, as part of their structure, specific antigenic determinants in the form of proteins or other antigenic moiety. Recognition of the diagnostic potential of such determinants for both experimental and applied investigations in plant pathology has resulted in a bewildering array of techniques being developed referred to as immunoassays. Immunassays are mainly used for identification of plant pathogens, diagnosis and detection of pathogenic organisms associated with plant disease, either within or isolated from affected plant tissues and direct quantitation of pathogens in plant tissues. Immunological techniques are also being used for various other purposes such as localization of pathogens within tissues (Brlansky *et. al.,* 1982; Hansen and Wick, 1993; Werres and Steffens, 1994) identification of specific antigens in electrophoretically separated components (Townsend and Archer 1983, Wakeham and White, 1996), quantitation of defense related proteins such as PR-proteins (Antoniw *et. al.* 1985, Kemp *et.al.*, 1999).

A short comprehensive review on the various serological techniques used in detection of plant pathogenic fungi has been presented below.

Serological detection of plant pathogenic fungi

Serological techniques such as immunodetection, immunoelectrophoresis, enzyme linked immunosorbent assays (ELISA) and immunofluorescence (IF) for detecting fungal pathogens have met with variable success. Various formats of ELISA have been developed, some of which are more appropriate for diagnostic laboratories. Available formats include multiwell, flow through, dipstick, dot-blot and tissue print assays. Multiwell ELISA has been used successfully to detect wide range of plant pathogenic fungi.

Phytophthora sp.

Species of the genus *Phytophthora* were differentiated by Burrell & Lilly (1966) using gel diffusion and immunofluorescence. The type and concentration of 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 hypae. Species-specific sera were obtained and proved useful and efficient for the identification of *P. cactorum. P. cinnamomi* and *P. erythroseptica.*

Studies on antigenicity of *Phytophthora* sp. by Merz *et. el.* (1968) revealed that cell-free extracts of mycelium grown on a glucose-$(NH_4)_2$ SO_4-fumaric acid medium containing β-sitosterol were more effective antigens than previous preparations. The stability of the cell-free antigens increased by the addition of ethylenediamine tetraacetate to the extraction buffer. Improvements and refinements were also made with the serological techniques by defining more precisely the buffers and templates in immunodiffusion tests. They used these methods to study the taxonomic relations, of six heterothallic species of *Phytophthora. P. cinnamoni* was serologically distinct, whereas two serological groups were evident among 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.* Therefore, these five species might best be incorporated into one *P. citrophthora.*

Gill and Zentmyer (1978) reported that species and isolates of *Phytophthora* species could be differentiated on the basis of soluble protein pattern. Soluble proteins from the mycelia of 30 isolates of *Phytophthora* were collected from 17 different host and from widely separated geographical location, and of five isolates of *P. cactorum,* when fractionated by disc electrophoresis, yielded 22 and 26 bands with different densities. The two species differed markedly and each exhibited its distinct, characteristic protein pattern enabling the authors to identify them. 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.*

In a similar study, it was shown that soluble proteins from 34 isolates representing 16 species of the genus *Phytophthora,* collected from different geographic locations and host plants when analysed by two-dimentional electrophoresis, were resolved into 100-200 protein spots with different densities. Although slight differences were occasionally observed in patterns of faintly-stained minor protein spots, those of densely-stained major protein spots were highly similar, if not identical, among isolates of the same species regardless of their geographic origin, original host plant, mating

type, or virulence race. These results suggest that the two-dimensional electrophoretic patterns are species-specific and may be a useful measure to differentiate and identify species of the genus *Phytophthora* (Masago *et. al.* 1989).

The extent of serological diversity in isolates of three species of *Phytophthora* viz. *P. palmivora, P. capsici* and *P. citrophthora* causing black pod disease of cocoa in India was also studied by immunodiffusion test (Chowdappa and Chandramohan, 2000). The three species of *Phytophthora* exhibited serological variation in their reaction with antiserum to either *P. capsici* or *P. citrophthora* in the agar double diffusion test. The serological reactions revealed that all the isolates of *P. palmivora, P. capsici* and *P. citrophthora* shared at least one common antigen. Two subgroups were distinguished among the isolates of *P. capsici* whereas all the isolates of *P. citrophthora* formed a homogeneous serological group. Thus, the results showed that serological technique can be used as additional criterion in distinguishing the three species of *Phytophthora* in support of morphological criteria.

Pscheidt (et.al. (1992) tested the sensitivity of a *Phytophthora* specific immunoassary 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. All *Phytophthora* isolates tested produced a positive reaction with the immunoassay kit. The lowest absorbance relative to other species were obtained from *P. cinnamomi* and *P. megasperma.* Variation in absorbance was high among isolates of *P. cinnamomi* but low among *P. cactorum.* Clinic samples with typical symptoms of *Phytophthora* infection produced a positive reaction with the immunoassay, as did pure cultures of *Phytophthora* sp. isolated from these samples. Cross reactions occurred with several *Phythium* sp. isolated from clinic samples and with several specimens infected with *Peronospora* sp. Others produced 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.

Hardham et. al. (1986) raised monoclonal antibodies to components on the surface of glutaraldehyde-fixed zoospores and cysts of an isolate of the pathogenic

fungus *Phytophthora cinnamomi.* Hybridoma supernatants were screened using an immuno fluorescence assay, and of 35 cell lines producing antibodies that reacted with the *P. cinnamomi* cells, 10 were selected and their specificities examined in detail. The monoclonal antibodies were found to possess a valuable spectrum of taxonomic specificities, and according to the authors, have revealed, for the first time, the presence of isolate-specific antigens on the surface of *P. cinnamomi* cells. The monoclonal antibodies were tested against six isolates of *P. cinnamomi,* six species of *Phytophthora,* and three species of *Pythium.* In addition to the isolate-specific monoclonal antibodies, species-specific and genus specific markers which are unambiguous in tests conducted so far have been obtained. The monoclonal antibodies have also revealed the presence of spatially restricted antigen on the surface of the zoospores. Some of these segregated antigens are species-specific and others are more general, occurring in all *Phytophthora* and *Pythium* species examined. All of the monoclonal antibodies promise to be of great assistance in investigations of the biology and taxonomy of *P. cinnamomi.*

In continuation of the previous study, Hardham and Suzaki (1989) studied the glycoconjugates on the surface of zoospores and cysts of *Phytophthora cinnamomi* using fluorescence isothiocyanate labelled lectins for fluorescence microscopy and flow cytometry, and ferritin-and gold labelled lectins for ultrastructural analysis. 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 suggested that the accessible saccharides, glycosyl or mannosyl residues predominate on the outer surface of the zoospore plasma membrane, vesiculate. These and other small peripheral vesicles quickly disappear. After the induction of ensystment, Con A was no longer localised close to the plasmamembrane but binds to the materials loosely associated in the cell surface. Quantitative measurements by flow cytometry indicated that the ConA-binding material was gradually lost from the cell surface. The cyst wall was weakly labelled, but the site of germ tube emergence stained intensely. During the first 2 min. after the induction of encystment, material that binds soybean agglutinin, *Helix pommatia* agglutinin, and peanut agglutinin appeared on the surface of the fungal cells. The distribution of this material, rich in galactosyl or N-acetyl-D-galactosaminosyl residues, was initially patchy, but by 5min the material evenly coated most of the cell surface.

Labelling of zoospores in which intercellular sites were accessible indicated that the soybean agglutinin binding material was stored in vesicles that lie beneath the plasma membrane. Quantitation of soybean agglutinin labelling showed that maximum binding occurs 2-3min. after the induction of encystment.

A set of five carbohydrate specific monoclonal antibodies (MAbs) were further used to probe the ultrastructure of the walls of the soybean pathogen *Phytophthora megasperma* f.sp. *glycinea* using a combination of immunoflurescence and immuno gold labelling techniques (Wycoff & Ayers, 1991). Results with β-1,3 glucan-specific antibodies suggested that β-1,3 glucans were present throughout the walls of both germ tubes and cysts, but were more prevalent in the outer portion. In addition, β-1,3 glucans on the surface of hyphal walls, but not cysts, were closely associated with other material, most likely protein, that sterically hinders antibody binding except to non-reducing terminal residues. An antibody whose epitope involved both β-1, 4 and β-1, 3 glucosyl linkages bound predominantly to the inner portion of the hyphal wall. However, fluorescent labelling with the antibody suggested that β-1, 4 linkages were present on the exterior of *P. megasperma* f.sp. *glycinae* wall as well. Staining with another antibody indicated that changes in wall composition occurred over 50-100μm from the hyphal tip, a greater distance than previously supposed.

Ten monoclonal antibodies were selected by Devergne *et. al.* (1994) from mice immunized with a highly purified elicitin secreted by *Phytophthora cryptogein*, termed cryptogein. This antibodies could be classified into five groups according to their cross-reactivity to heterologous elicitins from other *Phytophthora* species, from strict specificity (reacting solely with cryptogine) to broad reactivity (reacting with all four elicitins under study). When examined on BIA core (a real-time biospecific interaction analyser), these monoclonal antibodies were found to recognize at least 3 different epitopes on the cryptogein molecule. Their use in elecitin detection and quantification was optimized in several ELISA protocols. A mixed monoclonal-polyclonal antibody indirect DAS-ELISA procedure detected as little as 20pg. of purified elicitin per well (100μl). The four elicitins could be detected with the aid of one of couple of polyvalent reagents, whilst each one could be detected separately using appropriate monoclonal antibodies. These protocols have been used to detect elicitin secreted by *Phytophthora* spp. into culture medium as well as in plants following plant inoculation.

A dipstick immunoassay that is specific for *Phytophthora cinnamomi* was developed by Cahill and Hardham (1994) for use in soils. Azo dye detection of monoclonal antibody-labelled cysts attached to a nylon membrane provided a rapid, sensitive assay suitable for the field use. There was no cross-reaction with other *Phytophthora* and *Pythium* species in controlled environment assays or with soil or other organic matter that adhered to the membrane. The assay was as sensitive as a *Eucalyptus sieberi* baiting assay and when run together with the biting assay, was quantified for an infested soil water suspension from $2.5x10^2$ to $5x10^5$ zoospores per milliliter. The assay was used successfully to detect *P. cinnamomi* in a wide range of soil samples collected from beneath a diverse range of soil samples from a range of host species. According to the authors, there are several advantages to using the dipstic assay compared with traditional procedures; familiarity with *Phytophthora* taxonomy is not required, the assay can be performed by unskilled personnel; and soil rather than infected plant tissues can be assayed. Field testing of the assay showed that in kit form, it could be used as a reliable diagnostic tool to replace or augment current isolation and detection methods. The authors suggested that the dipstick assay should find broad use for the detection of *P. cinnamomi* in soil from forests and plant communities and in the horticultural and ornamental crops affected by this pathogen.

Alba and Devay (1985) detected cross reactive antigens in crude preparations from mycelia of *Phytophthora infestans* Race 1.2.3.4.7. with antisera for potatoes cv. King Edward and cv. Pentland Dell by using an indirect' ELISA technique. Results 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 are thermolabile and that they can be concentratrated by precipitation in the presence of 40% saturated ammonium sulphate (SAS). The results also revealed an antigenic disparity when 40% SAS from *P. infestans* Race 4, mycelia preparation was assayed with antisera for King Edward and Pentland Dell.

Amouzou-Alladaye et. al. (1988). raised antisera by injecting rabbits with mycelial protein extracts of one strain of *Phytophthora fragariae* which had a dilution end point of 1/64 in double diffusion and 1/512,00 in indirect enzyme-linked immunosorbent assay (ELISA). This serum could detect 11 different strains of *P.*

fragariae in pure culture and in naturally infected or inoculated roots. Although the sensitivities of direct double antibody sandwich and indirect ELISA were comparable, the direct double antibody sandwich 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 *P. fragariae* was detected reliably by ELISA several days before zoospores were found and before symptoms developed. Thus direct double antibody sandwich ELISA may be useful for early detection of infection and for the detection of latent infections of strawberry plants by *P. fragariae*.

Antiserum was raised against pooled mycelial suspensions from five isolates (designated Pf-1, Pf-2, Pf-3, Pf-10 and Pf-11) representing five physiologic races of *Phytophthora fragariae* by Mohan (1988). This antiserum detected homologous soluble antigens at protein concentration as low as 2ng/ml in enzyme linked immunosorbent (ELISA). 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 which is resistant to Pf-1, 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 corresponding extracts of plants inoculated with avirulent isolates. Hapil Ostara and Providence. The antiserum also detected *P. cactorum* infections.

Mohan (1989) also reported that 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 *Pythium* species were decreased by fractionating on an affinity column of Sepharose 4B bound to extracts of *Fragaria vesca* roots infected with *P. fragariae*. The affinity-purified anti-PfM retained its high cross reactivity with the various *Phytophthora* species tested. It 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 district patterns for *P. fragariae, P. erythroseptica* and *P. cactorum* : three serotypes were identified for the latter species. This antiserum might therefore prove useful in classifying *Phytophthora* species. Polyclonal antibody, raised in 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 micro organisms found on potato (Harrison et. al. 1990) *P. infestans* mycelium in leaf tissue was readily detected by ELISA using either the plate-trapped antigen or $F(ab^1)_2$ 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.

Benson (1991) compared two commercial serological assay kits to a culture plate method for detection of *Phytophthora cinnamomi* in root samples from inoculated azaleas. Both the multiwell E kit and the rapid assay F kit detected *P. cinnamomi* in azalea roots beginning 1 wk after inoculation. Agreement between immunoassay kits and culture plate results for detection of *P. cinnamomi* was most consistent beginning 3-4 wk after inoculation. Root symptoms, but not foliar symptoms, of *Phytophthora* root rot were evident during this period. There was a positive corelation 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. Although colour reactions in the rapid assay detectors became increasingly darker after completion of the test, results after 5min were as reliable as those after 60min, since readings for uninoculated controls used to determine test thresholds also increased with time. The mutiwell 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 value that were unconfirmed by culture plate. The rapid assay kit detected *P. cinnamomi* was easy to use, and gave results in short time.

A polyclonal, enzyme-linked immunosorbent assay of *Phytophthora infestans* has been developed by Beckman *et. al.* (1994) for use in the determination of fungal biomass during the early stages of infection of tuber disc of *Solanum tuberosum.* By optimizing the dilution of sample extracts and the dilution of primary anti *P. infestans* antiserum, quantification of the biomass of *P. infestans* in zoospore inoculated tuber disc could be achieved by 8-18 h after inoculation. Differences in growth between avirulent and virulent isolates of *P. infestans* on the resistant potato cv. kennebec were quantified by 32-48h after inoculation. Together with a comparison of 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. On the susceptible host, however, the avirulent isolate appeared more aggressive than the virulent isolate. These results demonstrated that 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 response.

MacDonald and Duniway (1979) investigated the use of fluorescent antibodies to study the survival of *Phytophthora megasperma* and *P. cinnamomi* zoospores in soil. For this they added zoospore cyst of the pathogens to non-sterile soil and its matric potential was adjusted to predetermined values. The infested soils were held under laboratory and field conditions and periodically sampled to determine cyst viability and soil water potential. During sampling soil containing cyst was suspended in water and aliquots were placed on a selective agar medium to allow germination of viable cysts. After germination, the cysts were stained by an immunofluorescence detection technique and counted. Under laboratory conditions, zoospores cysts of *P. megasperma* survived no longer than 3 wk at water ranging from 0 to –15 bars, and under field conditions they survived no longer than 2 wk. Zoospores cyst of *P. cinnamomi* survived no more than 3 wk in soil at water potentials of 0 to –1 bar under either field or laboratory conditions. However, at water potentials of –5 or –15 bars, zoospores cysts of *P. cinnamomi* survived upto 6wk in the laboratory. In a nonirrigated field plot, in which soil water potentials ranged from –7 to less than –100 bars, cysts of *P. cinnamomi* survived for 8-10wk. Under more controlled humidity

conditions, zoospore cysts of *P. cinnamomi* proved relatively tolerant of dessication; many survived for 1 wk in soils that dried to water potentials as low as –75 bars. Compared to *P. cinnamomi,* zoospore cysts of *P. megasperma* were much more sensitive to desiccation; most were killed within 1 wk in soil dried to water potentials of –20 bars or less.

The utility of immunofluerescent antibody technique for detecting *Phytophthora* in soil was also demonstrated by Mitsuo (1990). But the autofluorescence and the nonspecific staining of soil particles interfere with the detection of the fungi in soil. They reported that the pretreatment of the samples with gelatin-rhodamine conjugate prevented the samples from the autfluorescence and the non specific staining and therefore permitted the immunofluorescent antibody staining in soil. Stained *Phytophthora* was easily detected on yellow orange background.

***Ceratocystis* sp.**

Using the serological techniques of agglutination, gel diffusion and immunofluorescence, Amos and Burrell (1966) identified eight species of the genus *Ceratocystis* : *C. fagacearum, C. adiposa, C. ulmi* and *C. ips.* All three serological techniques proved to be applicable in the identification of these fungi. It was found necessary to absorb selectively cross-reacting antibodies firm the sera to make them species-specific. The immunofluorescence technique proved to be the most useful in differentiating among these species. Although all of the species could be shown to have serological differences, no such differences could be seen between the A and B compatibility types of *C. fagacearum.*

***Fusarium* sp.**

A strain-specific antiserum was raised by Hornok and Jagicza (1973) against the mature hyphae of *Fusarium culmorum* (W.G. Smith) Sacc. 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. There was a significant difference in the intensity of fluorescence between the mycelia of the homologous *F. culmorum* strain and those of heterologous *F. graminearum* and *F. culmorum* strains. All the other *Fusarium* sp. 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 for *F. culmorum* and *F. graminearum* was suitable to separate these species from other fungi. Morphological examination and culturing processes were not necessary because mycelia sufficient for fluorescent antibody staining usually grew out from infected plant material on to a microscope slide n 48-72 hours.

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

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 accurate ELISA for the homologous strains was developed by using the antiserum with β-D-galactosidase-labeled 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.

Rataj-Guranuwska and Wolko (1991) compared the extracts from *Fusarium oxysporum* (F.o.) and *F. oxysporum* var. *redolens* (F.o.r.) isolates by means of electrophoresis and crossed immunoelectrophoresis. The polymorphism of five 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.

An enzyme-linked immunosorbent assay (ELISA) for isomarticin, a naphthazarin toxin produced by *Fasarium solani* was developed. A carbodiimide procedure was used 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 toxin were detectable at less than 10 ng/per well in ELISA plates. The assay was specific for napthazarins. The cross reactivity for a number of phenolic compounds, including the closely related naphthoquinones, was three orders of magnitude less sensitive (Phelps *et. al.* 1990).

Naphthazarin toxins of *Fusarium solani* were also detected by competitive ELISA analysis in xylem fluid of roots rotted by *F. solani* and symptomless scafold roots and branches of healthy-appearing and diseased citrus trees in ridge and flatwood Florida groves by Nemee and Charest (1991). Studies concentrated on blight, a wilt disease with an undefined cause and etiology, to determine if *F. solani* is a causal factor of the disease. Healthy-appearing roots of trees with blight symptoms in six groves contained up to 11.4 times toxin than roots of healthy trees in the same groves. In blight-diseased trees from these groves, median toxin values per root and percentage of roots positive for toxin were higher than for healthy trees. Rotted roots from blight-diseased trees in the two groves contained 112 and 3.4 times more toxin than healthy appearing roots from the 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 in healthy-appearing roots of healthy tangerine and sweet orange trees on *Citrus limon* 'Milam' 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 root infected by *F. solani,* immunocytochemical localization of Naphthazarins reveled it to be 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

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electron-lucent areas. The presence of naphthazarin toxins in blight-diseased trees as well as those with other diseases suggests the non-specificity of *F. solani* pathogenic activity on various rootstocks. Therefore, *in situ* toxin concentrations high enough to trigger pathogenic effects in susceptible rootstocks may be required to cause blight.

Preparations of antigens from four varieties of *Gossypium hirsutum* and isolates of *Fusarium* and *Verticillium* species were compared for common antigen by Charudattan and DeVay (1972). At least one antigenic substance was common among the varieties of cotton and isolates of *Fusarium oxysporum* f.sp. *vasinfectum, F. solani* f.sp. *phaseoli, Verticillium alboatrum* 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* (non-pathogenic) 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 present in heterologous reactions between the fungal and cotton preparations. The common antigenic determinant shared by cotton and fungal isolates does not appear to be related to the severity of wilt symptoms, but it may affect host pathogen compatibility during the process of root infection.

A common antigen from conidia of *F. oxysporum* sp. *vasinfectum* that cross-reacted with antiserum to cotton root tissue antigens, was further purified by Charudattan and Devay (1981). They reported that 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 condia 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.

In a further study, major cross reactive antigenic substance (CRA), common to cotton (*Gossypium hirsutum*) and certain fungal parasites of cotton roots, was isolated and purified by DeVay and Wakeman (1981) to homogenity from conidial cultures of

Fusarium oxysporum f.sp. *vasinfectum* (F.O.V). Agar gel double diffusion tests indicated the presence of CRA not only in F.O.V. 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 epidermal 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.O.V. 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.

Chakraborty *et. al.* (1997) also determined the presence of CRA between soybean and *Fusarium oxysporum*. They performed pathogenecity test of *Fusarium oxysporum* on ten varieties of soybean which revealed Soymax and Punjab-I to be most resistant while JS-2 and UPSM-19 were most susceptible. Antigens were prepared from the root of all the ten varieties of soybean and mycelium of *F. oxysporum*. Polyclonal antisera were raised against the mycelial suspension of *F. oxysporum* and root antigen of the susceptible cutivar UPSM-19. Cross reactive antigens shared by the host and pathogen were detected first by immunodiffusion. The immunoglobulin fractions were used for detection of cross-reactive antigens by enzyme-linked immunosorbent assay. Antigens of susceptible cultivars showed higher absorbance values when tested against the purified anti *F. oxysporum* antiserum. Antiserum produced against UPSM-19 showed cross-reactivity with the antigens of other cultivars. Indirect staining of antibodies using fluoreciene isothiocyanate indicated that in cross reaction of roots of susceptible cultivar (UPSM-19) cross-reactive antigens were concentrated around xylem elements, endodermis and epidermal cells, while in the resistant variety, fluorescence was concentrated mainly around epidermal cells and distributed in the cortical tissues. CRAs were also present in microconidia, macroconidia and chlamydospores of the fungus.

Antibodies raised to the ribosomes of *Fusarium moniliforme* and corn (*Zea mays* L.) were shown to be specific by the Ouchterlony double-diffusion test and sucrose density gradient analysis of the antigen antibody reaction. Extracts of total

ribosomes from pith of infected corn could be separated into host, pathogen and other organism(s) fractions by the use of antibodies and sucrose density gradient centrifugation. (Marshall and Partridge, 1981).

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 Linfield (1993). Antiserum raised to cell wal 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 cross-reactivity 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 the pathogen on selective medium.

Disease resistance in pea (*Pisum sativum* L.cv Alcan) tissue inoculated with macroconidia of the incompatible fungal strain *Fusarium solani* f.sp. *phaseoli* was correlated with increased levels of mitochondrial DNA (mRNA) homologous to cloned disease resistance response (DRP) 49 gene sequences (Allaire and Hadwiger, 1994). Antiserum developed against a fusion protein containing segments of native DRR 49 was used to study spatial and temporal localization of the protein in the inner parenchyma layers of immature pods inoculated with either *F. solani* f.sp. *phaseoli* or the compatible f.sp. *pisi*. The protein corresponding to DRR 49 was localized primarily in the nucleus in all treatments.

***Penicillium* sp.**

Aspergillus and *Penicillium* spp. were the predominant fungi isolated by Warnock (1973) in cultures from 5 samples of stored grains. Using inmmunofluorescent method, the presence of these fungi in most grains was confirmed but the amounts of mycelium involved were shown to be small. *Alternaria* spp. were not isolated from 3

of the samples but small amount of *Alternaria* mycelium were detected in grains of all the samples studied.

Fuhrmann *et. al.* (1989) raised antisera from a rabbit immunized with *Penicillum verrucosum* var. *verrucosum*. These antisera were characterised by immunofluorescence and by indirect enzyme-linked immunosorbent assay for their reactivity with 44 strains of moulds. Antigenically, *P. verrucosum* var. *verrucosum* (subgenus *Penicillium*) appeared to be similar to strains belonging to subgenus *Furcatum,* but strongly different from *Penicillium frequentans* (subgenus *Aspergillides*). Specific absorption of antibodies to antigens confirmed the existence of similar biochemical structures on *Penicillium frequentans, Aspergillus versicolor* and *Aspergillus fumigatus.* Immunological procedures may thus significantly contribute to refine the taxonomic classification of moulds.

***Verticillium* sp.**

Since ELISA enables quantitative measurement of antigen Casper and Mendgen (1979) used it to estimate *Verticillium lecanii* at different stages of infection in leaves of wheat heavily infected with yellow rust (*Puccinia striiformis*).

Phytotoxic protein-lipopolysaccharide (PLP) complexes were isolated from dialyzed culture fluids of a pathogenic strain of *Verticillium dahlie;* PLP complexes which lack phytotoxic activity were isolated from a mutant non-pathogenic strain of the fungus (Nachmlas *et. al.* 1982). A comparison of profiles of the wild-type and mutant PLP eluting from an Agarose A-5 column revealed quantitative differences between the two major protein peaks of each strain. The corresponding peaks of the 2 strains had similar molecular of 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 content. Antiserum prepared against the components of peak 1 from the pathogenic strain reacted with an antigen in extracts of *Verticillium* infected potato plant tissue which was apparently identical to a moiety produced by the pathogen in culture.

Geric *et. al.* (1987) detected *Verticillium dahliae* in cotton root tissue with indirect enzyme-linked immuno assay. A soluble protein extract of *V. dahliae* was

used to prepare a specific antiserum. The reaction of this antibody to the hyphae of *V. dahliae* was detected with an alkaline phosphatase antirabbit IgG conjugant that hydrolysed the substrate. Napthol. AS phosphate to a product that reacted with a diazonium salt, yielding a colored precipitate outlining fungal hyphae. The hyphae were readily observed on and in the root cortex of the host using a dissecting microscope. This technique allows 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.

Gerik and Huisman (1988) further studied the colonization of field-grown cotton roots by *Verticillium dahliae* by using a specific immunoenzymatic staining technique. Colony densities at distances more than 1cm from the tip were relatively constant. 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 towards 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.

Sundaram *et. al.* (1991) prepared antisera against purified mycelial proteins from *Verticillium dahliae,* the predominant fungus species in the potato early dying complex. The tested antisera against crude mycelial preparations of *Verticillium* spp. using 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. The antisera did not react with mycelial proteins from *Fusarium* spp. from potato and cotton, with a *Collectotrichum* 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.

***Sporothrix* sp.**

Ishizaki *et. al.* (1981) investigated the serological cross-reactivity of *Sporothrix schenkii* serum with various unrelated fungi by use of immunodiffusion tests. A rabbit

anti *S. schenkii* serum was obtained, which related with *Cladosporium werneckii, C. carrionii, C. bantianum, Coccidioides immitis, Phialophora jeanselmei, P. gougerotii, P. dermattidis, Fonsecaea pedrosoi, Aspergillus fumigatus, Histoplasma capsulatum* and *Trichophyton mentagrophytes,* but not with *Saccharomyces cerevisiae* antigens. The serological determinantes responsible for the cross-reactions were suggested to be D-galactosyl residue.

***Epichloe* sp.**

An anitiserum, prepared to homogenates of washed *Epichloe typhina* mycelium grown in a liquid medium was used in an enzyme-linked immunosorbent assay (ELISA) to detect antigens of the fungus in tall fescue (*Festuca arundinacea*) tissue sample by Johnson *et. al.* (1982). With ELISA they could detect as little as 100 ng of freeze-dried *E. typhina* mycelium per millilitre, and could detect *E. typhina* in individual seeds of tall fescue. Of 14 fungal genera tested including *Acremonium, Claviceps, Helminthosporium, Pythium, Rhizoctonia, Sclerotium,* all showed reactivity less than 0.1% that of *E. typhina.*

***Eutypha* sp.**

Antisera were made by Gendloff *et. al.* (1983) 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. Both antisera showed low specificity, but specificity was improved by cross adsorption of the RITC conjugated cell wall antiserum with *Phomopsis longicolla.* Woody cross section 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 serum then stained with RITC-labeled goat anti-rabbit gamma globulin. Both procedure specifically stained hypae in wood sections. Hyphae stained indirectly in woody vine sections showed a much brighter fluorescence than the analoges hyphae stained by the direct method. Fungi of some species that restained' hyphae strongly with the direct method on glass slides did not always react when stained indirectly in woody vine sections in which they were growing.

***Macrophomina* sp.**

Chakraborty and Purkayastha (1983) raised antibodies against *Macrophomina phaseolina* (isolate MP.) antigens and roots of soybean cultivars Soymax and UPSM-19 which were susceptible and resistant respectively to charcoal rot disease. These antisera were used in agar gel double diffusion tests for the presence of common antigens between isolates of *M. phaseolina* and soybean cultivars. Immunoelectrophoretic tests revealed that four antigenic substances were common between the susceptible soybean cultivars and isolates of *M. phaseolina* but no common antigens were detected between resistant cultivars and *M. phaseolina.*

Antigens from two isolates of *Macrophomina phosiolina* (Tassi) Gold, a pathogen of groundnut (viz. *Cortieium sasakii* Shivai (Matsumoto), *Colletotrichum lindemuthianum, C. corchori* (Pagvi and Singh) and five cultivars of *Arachis hypogea* L. were also compared by immunodiffusion, immunoclectrophoretic, and crossed-immunoclectrophoretic techniques against *M. phaseolina* antisera for the presence of cross reactive antigens. Common antigens were found among the susceptible cultivars of groundnut and two isolates of *M. phaseolina* but not between non-pathogens and groundnut cultivars.No antigenic similarity was found between nonpathogens and *M. phaseolina* isolates. Crossed-immunoelectrophoretic tests confirmed that at least one antigen was common between cv. J-II and cv. TMV-2, cv. Kadiri 71-I and cv. TMV-2, and cv. Kadiri 71-1 and isolates of *M. phaseolina.*(Purkayastha and Ghosal, 1987).

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 phaseolina* (Chakraborty and Purkayastha, 1987). Glyceollin production also increased significantly after induction of resistance by sodium azide treatment. Cross reactive antigens were detected in purified preparations from mycelia of *M. phaseolina* with antisera of soybean roots by immunodiffusion and immunoelectrophoretic tests. An antigenic disparity was noticed in the susceptible cultivar (cv. Soymax) after chemical induction of resistance.

Detection and quantification of *Macrophomina phaseolina,* causal agent of charcoal/ dry root rot disease in many crop plants, was carried out by Srivastava and

Arora (1997) using the ELISA serological technique. Polyclonal antisera raised against soluble extracts of mycelium, the residual water insoluble mycelial materials or ribosomal proteins were evaluated for specificity and cross-reactivity with 16 common soil fungi by ODD and DAS-ELISA. Soluble and cell wall antisera exhibited strong cross reactivity with most of the fungal isolates. Ribosomal antibodies were less reactive to common soil fungi except *Fusarium oxysporum ciceri.* Mycelial antigens of *M. phaseolina* on chick pea roots were detectable with DAS-ELISA at a minimum concentration of 10 ng g^{-1} at 1:100 root: buffer dilution. Quantitative estimation of *M. phaseolina* on roots was evaluated by ELISA under different temperatures and moisture conditions, and in soil amended with a potential antagonist (*Trichoderma harzianm* -25-92). A significant reduction in ELISA values was observed in *T. harzianum* amended treatments. This method may be useful for detection and rapid screening of *M. phaseolina* under different environmental contidions.

***Ganoderma* sp.**

Antisera was prepared against the antigen of the pathogen *Ganoderma lucidum* causing foot rot of betelnut. (Reddy and Ananthanarayanan, 1984). This antiserum obtained appeared to be specific to the *G. lucidum.* Stained preparations of the G. *lucidum* isolates of betelnut fluoresced brilliantly on glass slide and no such fluorescence was observed with the other fungi studied. Root sections of all the affected palms and 5% of apparently healthy betelnut palms showed mycelim which emitted flurorescence. Authors suggested that the presence of *Ganoderma lucidum* in roots of betelnut can be detected by the induction of fluorescent antibodies.

***Phoma* sp.**

Antisera to *Phoma exigua* var. *foveata* and var. *exigua* were prepared by injecting rabbits and mice with protein solutions from mycelium (Aguelon and Dunez, 1984) Specific antibodies were isolated and immuno enzymatic techniques (double antibody sandwich ELISA and indirect ELISA) were used to test for the fungus in inoculated tubers and sprouts and stems grown from these tubers. The fungus was detected in these different tissues, with var. *foveata* being more aggressive, demonstrated the applicability and sensitivity of the technique. 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 antibodies from mouse ascite liquid which suggest the possibility of producing specific monoclonal antibodies.

***Phaeolus* sp.**

In liquid culture fungus *Phaeolus schweinitzii,* which causes a root and butt-rot of conifers, secretes a number of species specific and strain-specific polypeptides which are detectable by dodecyl sulfate polyacrylamide gel electrophoresis and isoelectric focussing (Dewey *et. al.* 1984). Indirect immunofluorescence microscopy was used to detect the binding of species specific antisera to these antigens 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 filtrates material and walls of mycelia, chlamydospores and basidiospores. Indirect immunofluorescence, performed by using antisera to culture filtrate molecules has been used to demonstrate the presence of mycelium, and on occasions chlamydospores in naturally and artificially infested soil samples. This makes possible identification of the kind of propagule most likely to be the source of field isolates of the organism. This information, which can not be obtained by using selective media, strongly suggests that the pathogen can survive saprophytically in the soil.

***Acrocylindrium* sp.**

Immunodiffusion, immunoelectrophoretic and cross immunoelectrophoretic 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* (Purkayastha and Ghosal, 1985). One precipitin band was observed when the antigenic 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.* Cross-immunoelectrophoretic tests confirmed that there was a common antigen between Mahsuri and Jaya, and between Mahsuri and CR-125-42-1. The precipitin band between the antigen preparation of Jaya and *A. oryzae* was found to be similar.

***Botrytis* sp.**

Ala El-Dein and El-Kady (1985) used crossed immunoelectrophoresis (CIE) with an intermediate gel and CIE with antibody absorption in situ techniques for comparison of antigenic structures of *Botrytis cinerea, B. tulipae, B. paeoniae* and *B. allii* isolates. Antisera against antigen of these isolates gave 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. Botrytis alli* had less common antigens than the other species.

Thin section of leaves of *Vicia faba,* uninfected were infected with two antisera, one prepared against the surface components of *B. cinerea* and the other against the timbriae of the smut fungus *Ustilago violacea.* Then a suspension containing protein A gold complexes was applied to the infected and uninfected sections. A full range of serological control treatments included use of preimmune serum. Treatment with either antiserum resulted in heavy gold labelling of host cells in infected tissue but not of cells in healthy tissue. In many cases the labelled host cells were not penetrated by fungus and were some distance from the nearest hypha. Treatment of infected host cells with preimmune serum did not result in gold labelling. Two hypotheses were proposed for these results. First fimbrial protein, either as intact fimbriae or as dissociated subunits, enters host cells well ahead of the hypae. Second infection by the fungus may stimulate a response by the host that includes synthesis of host proteins with antigenic regions similar to those of surface proteins of *B. cinerea* and those of fimbriae of *U. violacea.*(Svirecv *et. al.*, 1986).

Ricker *et. al.* (1991) detected water soluble antigens produced by *Botrytis cinerea* in spiked and naturally infected grape juice by using an enzyme immuno assay with an indirect format of antibody horseradish-peroxidase conjugate bound to polyclonal rabbit antibodies directed against *B. cinerea* (anti Bc IgG). Protein A purified gamma globulin from an early-bled antiserum (803-7), which reacted primarily with low molecular compounds present only in extracts of *B. cinerea* was used to specifically detect *B. cinerea* and quantify levels of infection in juice from infected

rape berries. Late bled, higher titer antiserum (803-19), which cross reacted with proteins and carbohydrate present in extracts from species of *Botrytis, Aspergillus, Penicillium* and *Uncinula* was used 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. Affinity purification of 803–19 IgG by using antigens from *Aspergillus niger* coupled to sepharose beads improved specificity of anti-Bc IgG to *B. cinerea* but decreased detection sensitivity to approximately 0.5% infection. Cross reactivity of all anti Bc IgG collection was consistently low with juice extracted from uninfected grape berries. In contrast, cross-reactivity of anti-Bc IgG with water soluble antigens extracted from sterile and reproductive structures of several fungi was negligible in early-bled antiserum and increased in subsequent collections. The increase in cross reactivity in late-bled antisera corresponded with an increase in the overall serum titres for anti-Bc IgG 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.

Salinas and Schots (1994) used whole conidia, their extracellular material and a putative cutin esterase isolated from conidia of *Botrytis cinerea* as antigen to raise monoclonal antibodies (MAbs) for the detection of condia of *B. cinerea.* Using immunofluorescence, three selected MAbs recognized conidia of 43 isolates of *B. cinerea* from hosts representing six countries. The percentage of conidia that fluoresced ranged from 50 to 100%. Intensity of fluorescence was related more to the MAbs than to the *Botrytis* isolate tested. MAbs showed no reaction with healthy gerbera 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.*

***Colletotrichum* sp.**

It was reported that agar gel double diffusion test confirmed the existence of common antigenic relationship between the susceptible jute cultivar and the foliar pathogen *Colletotrichum corchori.* Pathogenicity test of the isolate of *C. corchori* also revealed that it is virulent on cv. JRC 212 while JRC 321 is moderately resistant.

The precipitin reaction was observed within 24 hr. In case of jute, however, the reaction occurred only after 72 hr. Bhattacharya and Purkayastha (1985) suggested that the delayed response may be attributed to the slow rate of diffusion of the host antigen because precipitation was observed within 24hr. When reactivity was tested between the fungal antigen and its own antiserum.

In a further study, Purkayastha and Banerjee (1989) used six antibiotics as foliar spray on a susceptible soybean cultivar (Soymax) to induce resistance against anthracnose. In addition, common antigenic relationships between seven soybean cut tivars, their pathogens and non-pathogen were also studied using immunodiffusin, immunoelectrophoresis and indirect ELISA technique. Among the six antibiotics tested, cloxacillin and penicillin induced maximum resistance against anthracnose. Cloxacillin (100µg/ml) but not penicillin also altered the antigenic pattern of treated leaves. Cross reactive antigens were detected between susceptible soybean cultivars and the virulent strain of *C. dematium* var. *truncata* but no cross reactive antigens were detected between soybean cultivars and an avirulent pathogen (*C. dematium*) or non-pathogen (*C. corchori*). Results of immunodiffusion and immunoelectrophoresis 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.

Immunofluorescence studies of cutinase secretion by phytopathogenic fungi *Fusarium solani* f.sp *pisi* and *C. gloeosporiodes* were made to determine targeting of the enzyme in germinating spores (Podila *et. al.* 1994). Results indicated that the secretion of cutinase was directed towards the region that penetrates the host. In *F. solani* f.sp. *pisi*, which penetrates plant tissue without appressorium formation, cutinase was targeted to the growing tip of germinating spores. In *C. gloeosporioides,* cutinase secretion was directed to the infection peg that arises from the appressorium. Monensin, the carboxylic ionophore, inhibited secretion of cutinase and caused intracellular accumulation of cutinase in *F.* sp. *pisi*. Subcellular fractionation of monensin-treated cells indicated involvement of the Golgi in the secretion and targeting of cutinase by germinating spores of *F.* sp. *pisi.*

A study was conducted by Viswanathan (2000) on the possibility of identifying

resistance to red rot pathogen *C.falcatum* Went in sugarcane based on serological technique. Large number of genotypes were screened for red rot resistance at an early stage. A new serological technique, immunosorbent assay was developed based on antiserum developed against the fungus. Early detection of the pathogen well before the symptom development was possible in this technique. 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 a clear variation in disease resistance among them as in field testing. Further, this technique was found reliable to screen sugarcane genotypes for red rot resistance at an earlier stage.

***Mycena* sp.**

Antigenic determinants responsible for a precipitation line specific for *Mycena galopus* were partially purified using salt fractionation (with ammonium sulphate) and ion exchange chromatography by Chard *et. al.* (1985a). Fractionations were assessed by immunodiffusion and immunoelectrophoresis for the presence of the specific line. Proteins, with associated lipid and carbohydrate moieties, were detected in the antigenic fraction, which was used for further antiserum production. The new (F^{-}) antiserum was tested for its specificity to *M. galopus* by immunodiffusion.They further carried out immunofluorescence tests, involving 34 species of fungi, on an anti-*Mycena galopus* serum raised against a partially purified antigenic fraction. Cross reacting fluorescence was produced primarily by *Mycena* deuteromycete 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(Chard *et. al.* 1985b).

***Sclerotinia* sp.**

The adaptation of enzyme-linked immunosorbent assay for the detection of *Sclerotinia sclerotiorum* from both artificially and naturally infected host plants was described by Walcz *et. al.* (1985). The ELISA technique showed good specificity and sensitivity in detecting '*Sclerotinia* antigen' at a concentration as low as 10 ng ml^{-1}.

Jamaux and Spire (1994) also reported a serological test that allows the early detection of infection of young petals by *S.selerotiorum* an important pathogen of rapeseed. Two steps were required to obtain an antiserum sufficiently specific for *S. sclerotiorum.* Soluble mycelial extracts of *S. sclerotiorum* were used to produce the first generation polyclonal antiserum. This was not specific for *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 rapeseed 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 second generation antisera (S1 and s2), which were then tested by DAS-ELISA. Cross reaction with *B. cinerea* decreased with purification cycles of the immunogen whereas cross reaction with some unrelated fungi slightly increased *S. sclerotiorum* and *B. cinerea* were distinguished using antiserum S2.

***Ustilago* sp.**

Sections of leaves of *Nicotiana tabacum* L. infected with *Peronospora hyoscyami* De Bary f.sp. *tabacina* (Adam) Skalicky and of *Erythronium americanum* Ker. infected with *Ustilago heufleri* Fuckel were treated with an antiserum directed against the fimbriae of *U. violacea* Fuckel and other fungi (Day *et. al.* 1986). The sections were then treated with protein A-gold complexes to detect the presence and location of fimbrial antigens following transmission or scanning electron microscopy. Control preparations involved sections of uninfected leaves, as well as a range of serological control treatments. 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* sp. *tabacina* than for *U. heufleri* and was particularly high on the walls of the former species. Relatively high levels of labelling occurred over the cells of infected hosts, but little or none occurred over the cells of uninfected host tissues, or of the infected host tissues treated with a range of serological controls. This high level of labelling was not associated with specific host structures in *P. hyocyami,* but was frequently associated with the

chloroplasts in *U. heufleri.* The antigens detected inside the host plant cells appear to indicate that fungal fimbrial protein, either as polymerized fimbrial or as isolated subunits, can penetrate the host plasma membrane and therefore enter the host cytoplasm. An alternative possibility is that these antigens derive from host produced proteins synthesized as a result of infection. These results suggest the possibility that fungal fimbriae may play an important role in the molecular interaction between pathogen and host.

Cross-reactivity between antisera produced against fimbriae of *Ustilago violacea* (Pers) Fuck. (AU) or of *Rhodotorula rubra* (Demme) Lodder (AR) and cell surface proteins of two ascomycete fungi, *Aococalyx abietina* (Lagerberg.) Schlaepfer-Bernhard and *Ophistoma ulmi* (Buism.) Nannf., was revealed by means of dot-immunobinding and immunocytochemical methods (Benthamou *et.al.* 1986). Following treatment with antiserum AR, the walls, septa, and plasma membrane of *A. abietina* and *O. ulmi* cells (the latter either in culture or grown in elm wood sections) were appreciably labeled by gold particles, but the labelling intensity was always found to be greater over the plasma membrane. The fibrillar sheath surrounding cells of *A. abietina* reacted with antiserum A.U. while all other structures did not. No significant labelling with this antiserum occured over cells of *O. ulim* indicating that they either lacked these antigens or that those were more easily removed during the fixation process.

***Phomopsis* sp.**

Antiserum to freezed-dried powdered mycelium of *Phomopsis longicolla* soybean seed decay pathogen, was used by Gleason *et. al.* (1987) in an idirect ELISA and an immunoblot assay for detecting seed borne infections. Antigen of *P. longicolla* was detected by indirect ELISA in as little as 250 ng of dried mycelium per milliliter of extract. The antiserum reacted with mycelial preparations of *Diaporthe phaseolorum* var. *sojae* var. *caulivora* but showed comparatively little or no reaction when tested against seven other seed borne fungi. Extracts of whole seeds, or of seed coats, produced a non-specific background reaction that showed the specific reaction. *P. longicolla* could be detected in individual variety of symptomless infected seeds. A single infected seed in 20 could be readily detected by indirect ELISA of extracts of

seed coats. An immunoblot assay designated the seed immunoblot assay was developed to overcome problems with nonspecific interference in indirect ELISA. Mycelium of *P. longicolla* growing onto nitrocellulose paper from infected soybean seeds produced a conspicuous coloured blotch after the paper was assayed. Results of seed immuoblot assay (SIBA) for incidence of *P. longicolla* and *D.P.* var. *sojae* in halved seeds from 10 seed lots correlated ($P<0.001$) with agar plate bioassarys of the corresponding seed halves but not with incidence of symptomatic seeds. Indirect ELISA absorbance values for bulked samples of seed coat halves from the same 10 seed lots correlated weakly ($0.10>P>0.05$) with agar plate bioassays but strongly ($P<0.01$) with incidence of symptomatic seeds. Because SIBA detects only viable *P. longicolla* and ELISA does not discriminate between live and dead fungus, SIBA should be better indicator of pathogen activity on seeds after planting. This two types of serological assays apparently measure different aspects of this disease however and both may be useful for evaluating soybean seed lot quality.

Brill *et. al.* (1994) produced polyclonal antibodies in New Zealand white rabbits with cultiture filtrate and mycelial extract immunogen preparations from the soybean (*Glycine max*) fungal pathogen *Phomopsis longicolla.* The PAbs were purified to the immunoglobulin fraction and tested in indirect enzyme-linked immunosorbent assay (ELISA) and in double antibody sandwich-ELISA (DAS-ELISA). 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 immunizations and the activity became constant against most members of the complex at the same time. Reactivity to same cultures of *P. longicolla* was undetectable following the fourth and fifth immunizations, whereas reactivity to all of their other cultures of the complex remained high.

***Ophiostoma* sp.**

The surface deposition and accumulation of crato-ulmin by the aggressive isolates *Ophiostoma ulmi* were demonstrated by Svirecv *et. al.* (1988) using polyclonal

antiserum directed against cerato-ulmin, 100-150 nm protein A-gold particles, and a scanning electron microscope. The protein A-gold complex was present on the fungal surface in areas containing the toxin cerato-ulmin (CU). The gold level was present on the surface of the vegetative hyphae, synnemata, synnematal spores, perithecia and ostiolar hair of the aggressive isolates of *O. ulmi.* The protein A-gold level was either evenly distributed in 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 VA of *O. ulmi.*

***Pseudocercosporella* sp.**

Unger and Wolf (1988) presented an indirect ELISA for quantitative detection of *P. herpotrichoides* infections in wheat. All tested isolates of the virulent varieties *P. herpotrichoides infections, P. h.* var. *acuformis* or the W and R-type react on a high level in the test, while the less virulent *P. anguioides* is assessed only with 40% and avirulent *P. asestiva* with 20% of the homologous reaction. 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 through the leaf-sheaths was clearly reflected by ELISA. The examination of 24 stembase samples from the field showed that the values assessed by ELISA correlate well also with the disease indices of naturally infected plant material.

A double-antibody-sandwich ELISA test has been developed by Priestly and Dewey (1993) for the detection of *Pseudocercosporella herpotrichoides* using a highly specific monoclonal antibody pH-10 as the capture antibody and genus specific rabbit polyclonal antiserum as the detector antibody. The assay recognizes extracts from plants both artificially and naturally infected with *P. hypotrichoides* givining at least three-fold higher absorbance values with extracts from *Pseudocercosporella* infected tissue than with extracts from healthy tissues or from tissues naturally infected with *Microdochium nivale, Rhizoctonia cerealis* or material artificially inoculated with *P. anguioides.* The assay tested positively against all isolates of *P. herpoterichoides,* including both W-type and R-type isolates. In this assay system, extraction of the

antigen from the stem bases of infected plants is a one-step process not requiring any dilution procedures. The assay can detect the pathogen in presymtomatic infected seedlings. The immunogen used to generate the specific monoclonal antibody and the rabbit antiserum was a mycelial extract from which the high-molecular-weight proteins and glycoproteins had been removed by ammonium sulphate precipitation. The high-molecular weight fraction was shown to contain cross-reactive antigens, it induced antiserum in mice that cross-reacted with the other stem-base fungi even at high dilutions. The monoclonal antibody pH-10 is an IgM antibody. Heat and periodate treatment of the antigen indicate that it is glycoprotein and that the epitope recognized by the antibody is a protein.

***Plasmodiophora* sp.**

An antiserum was made against *Plasmodiophora* brassicae, the causal agent of club root of cabbage (Lange *et. al.* 1989). A semipurified suspension of spores of *P. brassicae* was used as antigen, obtained by filtration and percoll gradient centrifugation of infected roots. Crude root suspension samples were bound to a nitrocellulose membrane and tested by a dot immunobinding 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 immunobinding processed 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 of *P. brassicae* did not react with surface antigens of resting spores of *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 immunobinding technique can be used as a routine test for detection of infection of *P. brassicae* in the host plant and in bait plants (used as indicator of soil infestation). The sensitivity obtained was within the range permissible for a routine test.

Polyclonal antisera were raised by Wakeham and White (1996) to whole (Coded : 16.2), and sonicated (Coded : 15/2) resting spores of *Plasmodiophora brassicae,* and to soluble components prepared by filtration 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 bloting the lower limit of detection with antiserum 15/2 was 1x10. This antiserum showed the greatest sensitivity in a dipstic assay, indirect ELISA and indirect immunofluorescence, for all of which there was a limit of detection of 1x10. The indirect ELISA was successful only after the substitution of alkaline phosphatase by protein A conjugated horseradish peroxidase. Of the assays tested, indirect immunfluorescence appears to be the most rapid and amenable assay for the detection in soil of low levels of resting spores of *P. brassicae*.

***Armillaria* sp.**

Antibodies to three isolates each of *Armillaria mellea, A. ostoyae, A. tabescens* and *Lentinula edodes* were isolated from eggs of immunized laying hens. The reactivity of each antibody preparation with the isolates was examined using an enzyme-linked immunosorbent asay (ELISA). The cross-reactivity of the antibody preparations to a given *Armillaria* species varied considerably when tested against isolates of other *Armillaria* species. Several antibody preparations were capable of distinguishing isolates of homologous species from isolates of heterologous species. The specificity of the antibodies present in eggs was dependent on time elapsed since immunization. Eggs laid 3 weeks after immunication with an *Armillaria* species isolate possessed antibodies that were most specific for isolates of that species. The enterogeneric cross-reactivity was found to be smallest with antibodies from egg laid 5 weeks or more after immunization. Preliminary results suggest that ELISA using chicken egg yolk antibodies will provide useful information in the taxonomy of *Armillaria* (Burdsall *et. al.* 1990).

***Leptosphaeria* sp**

Monoclonal antibody (MAB) LKe50 was developed against *Leptosphaeria* Korrae strain ATcc 56289. The antibody was capable of detecting *L. korrae* from culture and in naturally infected Kentucky bluegrass samples from three states (Nemeth

et. al.,(1990). In cross-reactivity testes using indirect ELISA, MAB LKc50 reacted positively to all 24 isolates of *L. korrae* screened, including strains from six states, from both Kentucky bluegrass and burmudagrass. MAb LKc50 reacted negatively to 38 of 42 isolates of related and nonrelated fungi and negatively to apparently healthy plants. The limit of detection was less than 2μg/ml of lyophilized mycelial homogenates. MAb LKc50 provides a means for rapid detection of *L. korrae* an ectotrophic root invading fungus that is difficult to identify using conventional methods.

***Myrothecium* sp.**

Antigens were extracted by Ghosh and Purkayastha (1990) from two resistant, three susceptible soybean cultivars and three strains of *M. roridum* a causal organism of leaf spot disease. Rabbit antisera were also raised against all three strains (M-1, ITCC-1143, ITCC-1409) of *M. roridum,* two susceptible cultivars (DS-74-24-2 and PK-327) and a resistant cultivar (UPSM-19) of soybean for analysis of cross reactive antigens. Result 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-immunoelectrophoresis.

***Venturia* sp.**

An immunocytochemical stain was developed by Young and Andrews (1990) to detect the basidiomycete *Athelia bombacina* in apple leaf litter. The polyclonal antibodies for *A. bombacina* were sufficiently specific that 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. Sections stained immunocytochemically showed that *A. bombacina* grews endophytically and epiphytically. The antagonist prevented neither growth of hyphae of *V. inaequalis* into the interior of 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 pseudathecia of *V. inaequalis.*

Pseudothecia in leaves with the antagonist did not mature further than the stage. of producing pseudoparaphyses, reaching on average of 84 μm in length. Pseudothecia in leaves without *A. bombacina* developed as in normally and were 108 μm long by 1 May. These data were confirmed by results from an abbreviated sampling by results from an abbreviated sampling scheme of McIntosh apple leaves during 1987-1988 and by observation of pseudothecial inhibition in crab apple leaves following delayed application of the antagonist.

***Acremonium* sp.**

Distribution of endophytic fungus, *Acremonium coenophialum,* with tissues of infected tall fescue (*Festuca arvudinacea*) can be determined with tissue print-immunoblot (TPIB); however, the accuracy of this method in determination of endophyte infestation levels of pastures and seed losts has not been evaluated (Gwinn *et. al.* 1991) Authors conducted a study to compare TPIB to other methods used for endophyte detection. Seed stocks of known endophyte infestation levels were tested 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 pasture. Therefore, accuracy of TPIB is comparable to other endophyte detection techniques and can be used for routine detection of endophyte in tall fescue tissue.

***Phialophora* sp.**

Daniel and Nilsson (1991) raised polyclonal antiserum against mycelial extracts from wood degrading soft rot fungus *Phialophora mutabilis.* In enzyme linked immunosorbent assay (ELISA), the antiserum reacted strongly to moderately with six soft rot *Phialophora* species. With exception of *Cerotocystis albida,* the serum reacted weakly or not at all with 11 other mold, and rot fungi occurring frequently in or on wood. The antiserum was cross-reacted strongly with antigens in extracellular filtrates from *P. mutabilis* cultures that contained about 40ng/ml of protein. Utrastructural and immunocytochemical studies on wood degraded by *P. mutabilis* showed specific localization of 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 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 for wood blocks with highest substrate losses and vice versa.

***Pythium* sp.**

A panel of monoclonal antibodies (MAbs) designated PA1 to PA8 has been raised by Estrada-gracia *et. al.* (1989) against cell surface components of zoospores and cysts of the pathogenic fungus *Pythium aphanidermatum.* The antibodies were selected on the basis of binding assays using indirect immunofluorescence. Four binding patterns were observed; PA1 labeled the entire zoospore surface including both flagella, in PA2 binding was restricted to the anterior flagella, PA3-PA6 bound to the adhesive cell coat secreted by zoospres during ensystment, and PA7 and PA8 labeled zoospores and the cyst cell wall. Electron microscopic immunogold labelling of zoospores showed that PA2 bound to the mastigonemes on the interior flagellun. The MAbs were tested for binding to zoospores and cysts of several isolates of *P. aphanidermatum,* and to zoospores and cysts of several species of *Pythium, Phytophthora, Aphanomyces,* and *Saprolegnia.* This results showed that the antigens recognized by MAbs PA1-PA6 were restricted to *P. aphanidermatum,* whereas those recognized by PA7 and PA8 occured on all species tested.

Shane (1991) compared methods for sampling turfgrass tissue for their effectiveness in monitoring *Pythium* blight epidemics with enzyme-linked immunosorbent assay (ELISA). Sample area consisted of marked strips on golf course fairways and tees with bentgrass and annual bluegrass naturally infested by *Pythium aphanidermatum.* Samples consisted of (1) whole plants picked by hand and assayed as whole plants, (2) whole plants sectioned into lower, middle and upper strata components; and (3) leaf clipping collected with a reel mower set at a 1.2cm cutting height. ELISA readings for mowed samples generally matched those for whole-plucked samples (r^2 values ranging from 0.457 to 0.601). Fluctuation in detectable *Pythium* antigens were most pronounced on the uppermost stratum compared with moderate to vary little change in ELISA readings for the two lower strata. Several episodes of

Pythium antigen increase were detected by ELISA assay 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. Antibody aided detection is useful for varification of diagnosis and determination of general *Pythium* population fluctuations, but current methodology is not satisfactory for advanced detection of blight epidemics.

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. Polyclonal antisera were raised against the ribosomes of *Pythium paddicun, P. iwayamai, Typhula incarnata* and *Microdochium nivale* and tested for sensitivity and specificity with ribosomes of these four pathogens, *Typhula ishikariensis,* and wheat. Using polyclonal antisera, ribosomes of *P. paddium* 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* spp. *Typhula* spp. *M. invale* and wheat. With indirect ELISA, ribosomes of *Pythium* species 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 rotten wheat leaf homogenates diluted upto 1:1000 or 1:10000. The indirect ELISA could not differentiate *P. paddicum* from *P. iwyamai* infected plants or *T. incarnata* from *T. ishikariensis* infected plants but could detect and differentiate snow mold fungi at the genus level in wheat plants. Western blot analysis with these antisera 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 sera would be useful to evaluate wheat plants for infection by *Pythium* spp., *T. incarnata T. ishikariensis* and *M. nivale*.

Conventional methods indicated that *P.violae* was most commonly isolated from carrot cavity spot samples from 14 UK sites. For one site the most frequently isolated species was *P. sulcatum.* Results of 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 by 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 negative.

White *et. al.*(1994) raised two monoclonal antibodies and three polyclonal antisera to cell wall / membrane fractions of *P.violae* and *P.sulcatum*. When screened with 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. Multivariate analysis methods were used to establish relationships between isolates on the basis of these profiles; isolates of the same species tended to group together. The affinity of these reagents for fungi other than members of the genus *Phytophthora* was low.

***Bipolaris* sp.**

Chakraborty and Saha (1994) reported that TV-18 was most susceptible and TV-26 most resistant among the 14 Tocklai varieties of tea tested against *Bipolaris carbonum.*Antigens from tea varieties, isolates of *B. carbonum* and nonpathogens of tea (*B. tetramera* and *B. setaria*) were compared by immunodiffusion, immunoelectrophoresis 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 (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 nonpathogens 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.

***Sclerotium* sp.**

Serological differences among three strains (266, 23, M) of *Selerotium rolfsii* were studied by Purkayastha and Pradhan (1994) along with strain pathogenicities and antigenic relationship with five groundnut cultivars. Changes in antigenic patterns of one of its host evs. AK-12-24 and disease intensity were noted after treatment with a systemic fungicide Kitazin. Results revealed that significant variation exists among the strains of the pathogen. The strain 266 was most virulent having antigenic relationship with susceptible cvs. (Gangapuri, J-11 and AK-12-24). Resistant cvs. JL-24 and ICGS-26, however, showed no antigenic relationship with fungal strains in either immunodiffusion or immunoelectrophoretic test. 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.

***Thielaviopsis* sp.**

Field isolates of *Thielaviopsis basicola,* the causal agent of black root rot of cotton (*Gossypium hirsutum*), were grown in Czapek-Dox broth amended with dialysed carrot extract (Holtz, 1994). Soluble protein extracts of chlamyolospores 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 gray cultural types of *T. basicola* and had negligible cross-reactivity with other soilborne fungi commonly found in the San Joaquin Valley of Callifornia cotton field soils. The minimum detection limit of ELISA was between 1 and 20ng of *T. basicolla* protein depending on the assay. *T. basicolla* 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.

***Exobasidium* sp.**

Blister blight of tea caused by *E. vexans* is one of the most destructive fungal diseases in Darjeeling hills. It causes enormous losses to Indian Tea Industry. Polyspecific antisera were raised against a plant cell supernatant fraction from

homogenized naturally blister infected leaf tissues (Variety AV-2) and subsequently the sensitized antisera were immunoprecipitated with healthy leaf antigens of AV-2 in order to separate antibodies unique to *E. vexans*. Immunoglobulin (IgG) was purified following ammonium sulphate fractionation and chromatography on DEAE-Sephadex Cross reactive antigens (CRA) were detected between the pathogen and susceptible Darjeeling tea varieties in immunodiffusion tests and enzyme-linked immunosorbent assay. Cellular location of CRA in cross-section of leaves was determined using indirect staining of antibodies with FITC-labelling. Specific and sensitive serological assays have been developed for early detection and diagnosis of the disease in Darjeeling and UPASI varieties of tea. Such rapid detection prior to the development of foliar symptoms will be helpful for making decision on management of blister blight which is the most threatening foliar fungal disease of tea (Chakraborty *et. al.* 1995).

***Pestalotiopsis* sp.**

Among the 12 varieties of tea tested against three isolates of *Pestalotiopsis theae,* causal agent of gray blight disease, Tean Ali-17/1/5A and TV-23 were found to be highly susceptible while CP-1 and TV-26 were resistant under identical conditions (Chakraborty *et. al.* 1995). Leaf antigens were prepared from all the tea varieties, three isolates of *P. theae* and a non-pathogen of tea (*Bipolaris 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 parasite. CRA were found among the susceptible varieties and isolates of *P. theae* and resistant varieties, *B. tetramera* and tea varieties or isolates of *P. theae.* Indirect staining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross reaction 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.*

Polyclonal antiserum raised by Chakraborty *et. al.* (1996), against the mycelial extract of *Pestalotiopsis theae* fractions were further purified by immonium sulfate frationation and chromatography on DEAE-Sephadex and the ammunoglobin fractions used for further tests. In enzyme-linked immunosorbent assay, antiserum dilution

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 25µg/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 up to 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 domonstrated that ELISA can be used for early detection of *P. theae* in leaf tissues even at a very low level of infection.

***Glomerella* sp.**

Chakraborty *et. al.* (1996) conducted varietal screening of tea (*Camellia sinensis*) with *Glomerella cingulata,* causal agent of brown blight disease with sixteen varieties released by Tocklai Experimental Station, Jorhat, Assam, following detached leaf, cut shoot and whole plant inoculation techniques. Among the tested varieties, TV-18 and Teenali 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 of *G. cingulata* (isolata GC-1). These antisera were used in immunodiffusion and immunoelectrophoretic tests as well as in enzyme liked immunosorbent assay (ELISA) to detect cross reactive antigen (CRA) shared between tea varieties and isolate of *G. cingulata.* 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. Indirect staining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross section of tea leaves (TV-18), the CRA was concentrated in epidermal cells, mesophyll tissue and vascular elements. Among a number of foliar fungal pathogens of tea *G cingulata* (Stoneman) Spauld and Schrenk which causes brown blight disease, is an important one. Polyclonal antisera were raised in separate white male rabbits against antigens prepared from

mycelia and cell wall of *G. cingulata* by Chakraborty *et. al.* (2000). Antigens were prepared from healthy artificially inoculated with *G. cingulata* and normally infected leaves of 5 UPASI varieties and DAC-ELISA performed. Pathogen could be detected in infected leaf extract at a concentration as low as 1mg/ml. Absorbance values of extracts of leaves inoculated with *G. cingulata* were higher than those inoculated with other pathogens and healthy extracts. Pathogenicity of *G cingulata* (Stoneman) Sparld and Schrenk, causal agent of brown blight disease, tested Chakraborty *et. al.* (2002) under identical conditions following detached leaf and cut shoot inoculation techniques against fourteen varieties of tea [Camellia sinensis (L.) O. Kuntze] revealed that CP-1 and TV-26 were resistant while TV-18 and Teen Ali-17/1/5A were susceptible. Polyclonal antisera were raised separately against antigen preparations from mycelia and cell wall of *G. cingulata* 9 isolate Gc-L) mycelia of *F. oxysporum* and leaf antigens of TV-18 and CP-1. Cross reactive antigen (CRA) were found among the susceptible varieties of tea and isolates of *G. cingulata* (G c-1, 3 and 3). Such antigens were not detected between resistant varieties of tea and isolats of *G. cingulata* (Gc-1, 2 and 3); non-pathogen (*Fusarrium oxysporum*) and tea varieties; isolates of *G. cingulata* and *F. oxysporum* and between non-host (*Glycine max*, *Cicer arietinum* and *Camellia japonicum*) and *G. cingulata.* Antisera raised against cell wall preparations gave better recognition than that against mycelial preparations as observed in ELISA test with antigens of tea leaves of different ages.

***Spongospora* sp.**

Walsh *et. al.* (1996) produced a polyclonal antiserum against spore balls of *Spongospora subterranea* f.sp. *subterranea* prepared from potato tubers which was able 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 concentration above 2000 spore balls per g soil than at lower concentration. 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 in such soils better than

the bioassy. The antiserm did not react with 30 other micro-organisms 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 attempt to improve the sensitivity of the serological detection through concentrating spore balls from field soils by sieving was unsucessful. Cross absorption of the antisera with uncontaminated field soils increased the sensitivity of detection of spore balls in spiked soil samples four-fold. The ability of the antiserum to discriminate contaminated field soils from 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 back ground fluoresecence was evident.

***Spodoptera* sp.**

Polyclonal antibodies were produced in mice against *Spodoptera exigua* (beet armyworm) larval hemolymph and hemocystes and against cell wall surfaces of hyphal bodies and hyphae of entomopathogenic hyphomycete *Nomuraea rileyi* (Pendland and Boucias, 2000). In addition to exhibiting strong activity against their original antigenic substrates, all of the antibodies cross-react extensively with other substrates. The hemolymph antibody binds to hemocytes and vice versa, and both antibodies cross-react extensively with other substrates. The hemolymph antibody binds to hemocytes and vice versa, and both antibodies cross react to the insect fat body basement membrane (extracellular matrix (ECM). Likewise, the antifungal antibodies cross-react with *S. exigua* hemolymph and hemocytes, especially the granutes that may contain ECM components, and with fat body basement membrane. These cross-reactivities are specific as indicated by negative controls in the microscopy and western blotting assays. Parallel labeling experiments using conA suggest that reactive epitopes contain mannose; however none of the antibodies bind to mannose residues of nonentomopathogenic *Candida albicans* or *Saccharomyces cerevisiae* yeast cells.

Thus, these cross-reactivities suggest the host minicry expressed by surface components of entomopathogenic fungi represents and important pathogenic determinant.

***Fomes* sp.**

Polyclonal antibody (PAb) was raised in New Zealand male rabbits against immunogen preparations from mycelial extract of *Fomes lamaoensis* (Murr.) Sacc and Trott., causal agent of brown root rot disease of tea (Chakraborty *et. al.* 2001). Eight blood samples were collected by marginal ear vein puncture from rabbits and IgG were purified using DEAE cellulose. Immunodiffusion tests were performed inorder to check the effectiveness of mycelial antigen preparations of *F. lamaoensis* for raising PAbs. Optimization of PAbs were done using indirect enzyme linked immunoassay (ELISA) Increased activity of PAbs against *F. lamaonensis* could be noticed from second bleeding, which continued upto fourth bleeding. Root antigens prepared from healthy and artificial inoculated (with *F. lamaoensis*) tea plants (TeenAli-17, TV-18, TV-22, TV-26, TV-27, TV-28, TV-30, S-449, BSS-2) were analysed following direct antigen coated enzyme linked immunosorbent assay (DAC-ELISA). Such format was also used to detect the pathogen in infested soil. Young mycelial of *F. lamaoensis* gave bright fluorescence in indirect immunofluorescence tests using PAbs and FITC-conjugates of goat specific for rabbit globulin. Such immunological assays developed for detection of *F. lamaoensis* in rhizosphere of tea plantation can enable disease prevention at an early stage.

***Ustulina* sp.**

Chakraborty *et. al.* (2001) raised polyclonal antibodies (Pabs) against mycelial and cell wall antigens of *Ustulina zonata* (Lev.) Sacc. causing charcoal stump rot of tea root. PAbs were purified in DEAE-cellulose column. The effectiveness of antigens preparation for raising PAbs were checked following immunodiffusion test Optimization of Pabs were done using indirect enzyme linked immunosorbent assay (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. Indirect immunofluorescence using PAbs and FITC-conjugates of goat specific for rabbit globulin were assessed for their potential

to detect mycelia and spores in soil. Of the assays tested indirect immunofluorescence appears to be most rapid assay for detection of pathogen in soil. Such immunological methods developed for early detected of *U.zonata* from soil and tea root tissue will be useful for the proper management of this disease.

Wide range of immunoassays available to the plant pathologist have been illustrated and factors to be considered in selecting the most appropriate technique have been indicated. Each has a role to play but there is no doubt that the development and adoption of highly sensitive assays such as ELISA, dot blot, immunofluorescence have transformed serology over the last two decades and revolutionized the plant pathologist's approach to the immunological identification of pathogens and the diagnosis of plant disease.