LITERATURE REVIEW

An advancing area in the study of host-pathogen interaction is the case of serological techniques to determine host pathogen interactions. With the introductio of ELISA for assaying plant viruses, serological methods are being used regularly in agricultural research an practice (Clark and Adams, 1977). Though, application of immunological technique for detection of fungi and bacteria came later then viruses, diagnostic kits are now available for detection of specific fungi in diseased tissues and soil. At present, Immunoassay are also being used for various other purposes such as ocalization 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 in different plant tissues and soil has been presented below.

A. Root tissue

Preparations of antigens from four varieties of *Gossypium hirsutum* and isolates of *Fusarium* and *Verticillium* species were compared for common antigens by Charudattan and DeVay (1972). At least one antigenic subtance 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 non-pathogenic 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 common in heterologous reactions between the fungal and cotton preparations. The common antigenic determinants shared by cotton and the fungal isolates does not appear related to the severity of wilt symptoms, but it may affect host-pathogen compatibility during the process of root infection. An antigen from conidia of *Fusarium oxysporum* f. sp. *vasinfectum* that cross-reacted with antiserum to cotton root tissue antigens was also purified and partially characterised by Charudattan and DeVay (1981). In agar-gel double diffusion tests, one precipitin band was formed when antiserum to

cotton antigens was reacted with crude fungal antigens, or when antiserum to crude fungal antigens was reacted with cotton antigens. Cross reactive antigen (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.

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

DeVay *et. al.* (1981) also reported that agar gel double-diffusion tests indicated the presence of CRA not only in *F. oxysporum* f. sp. *vasinfectum* and in cotton roots and seed but also in *Thielaviopsis basicola.* Indirect staining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross-sections of roots, cut near or just below the root hair zone the CRA was concentrated mainly around xylem elements, the endodermis and 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 plasma lemma. Treatment of conidia and mycelia of *F. oxysporum* f. sp. *vasinfectam* 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 and Purkayastha (1983) found serological relationship between *Macrophomina phaseolina* and soybean cultivars. Using antisera raised against antigens of *Macrophomina phaseolina* (isolate MP_1) and roots of soybean cultivars Soymax and UPSM-19 which were susceptible and resistant respectively to charcoal rot disease, agar-gel double diffusion test were carried out for the presence of common antigens between isolates of *M. phaseolina* and soybean cultivars. Immunoelectrophoretic tests revealed that four antigenic subtances were common between the susceptible soybean cultivars and isolates of *M. phaseolina* but no common antigens were detected between resistant cultivars and the fungus.

Pathogenicity of *Macrophomina phaseolina* was also tested on ten cultivars of soybean by Purkayastha and Chakraborty (1983). Soymax and UPSM-19 were most susceptible and resistance cultivars, respectively. Infected resistant cultivars produced significantly more glyceollin (phytoalexin) than the infected susceptible ones. Agar-gel double diffusion tests exhibited common antigenic relations between susceptible soybean plants and *M. phaseolina.* Immunoelectrophoretic studies revealed that four common antigenic substances were present between pathogen and susceptible cultivars, whereas no common antigenic substance was observed between resistant cvs and the pathogen (three isolates tested).

Dewey *et. al.* (1984) reported that in liquid culture, the 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 sodium dodecyl sulfate-polyacrylamide gel electrophoresis and isoelectric focusing. 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 extracullular culture filtrate 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. Authors suggested that this method could be used for identification of the kind of propagule most likely to be the source of field isolates of the organisms; this information, which cannot be obtained by using selective media, strongly suggests that the pathogen can survive saprophytically in the soil. In contrast, isolated mycelial cell wall preparation did not prove to be a suitable source of immunogenic material for these studies.

The presence of *Ganoderma lucidum* in roots of betelnut is detectable by the induction of fluorescent antibodies. This provides an interesting practical application of the technique which is developed by Koti Reddy & Ananthanarayanan (1984).

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. 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 (Chakraborty and Purkaystha, 1987). Purkayastha and Ghosh (1987) also prepared the antigens from two isolates of *Macrophomina phaseolina* (Tassi) Gold, a pathogen of groundnut, four non-pathogens of groundnut (viz. *Corticium sasakii* Shirai (Matsumoto), *Colletrichum lindemuthianum, C. corchori* (Pagvi and Singh), and *Botrytis ailii*), and five cultivars of *Arachis hypogea* L. These were compared by immunodiffusion, immunoelectrophoretic, and crossed-immunoelectrophoretic techniques 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 non-pathogens 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 71-1 and cv. TMV-2 and cv. Kadiri 71-1 and isolates of *M. phaseolina.*

Working with another pathogen of cotton, Gerik and Huisman (1988) studied colonization of field-grown cotton roots by *Verticillium dahliae* by using a specific immunoenzymatic staining technique. Colony density of *Verticillium dahliae* increased with distance from the root tip, with the maximal density occurring more than 1 cm from the root apex. Colony densities at distances more than 1 cm from the tip were relatively constant. The mean colony length of *V. dahliae* was 7.3mm., and increased colony length was correlated with distance from root apices. Hyphae of *V. dahliae* were present through the entire depth of the cortex, and were greatest in the interior of the root cortex at the surface of the vascular cylinder. The colony appearance was consistent with growth of hyphae from the root surface toward the stcle. Hyphae of *V. dahliae* also were found with in numerous cortical cells. Colonies of *Fusarium oxysporum,* similarly stained, were found to be mostly confined to the root surface and the outer cortex.

Antiserum was raised against pooled mycelial suspensions from five isolates (designated Pf1, Pf2, Pf3, Pf10 and Pf11) representing five physiologic races of

Phytophthora fragariae (Mohan, 1988). In enzyme-linked immunosorbent assay (ELISA), this antiserum detected homologous soluble antigens at protein concentrations as low as 2ng/ml. 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. ananssa* 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 Pf1, 2 and 3 but susceptible to Pf10 and 11, reflected this differential response in ELISA : the absorbance produced by extracts of plants infected with virulent isolates was significantly higher than that obtained with the corresponding extracts of plants inoculated with avirulent isolates. Cultivars Hapil, Ostara and Providence were found to be susceptible to all isolates in this study : the corresponding root extracts were also positive in ELISA. The antiserum also detected *P. cactorum* infections. Nevertheless, the authors concluded that the ELISA test described should prove valuable in screening certified strawberry stocks.

Yuen *et. al.* (1988) developed a double antibody sandwich indirect enzyme-linked immunosorbent assay (ELISA) for the detection and quantification of *Pythium ultimum.* A polyclonal antibody produced to cell walls of *P. ultimum* was used as the capture antibody, while a *P. ultimum* specific monoclonal antibody (MAbE5) was used for recognition of the fungus. In the ELISA, culture extracts of 7 isolates of *P. ultimum* exhibited strong positive reactions, where as none of the 37 isolates of other *Pythium* spp. and fungal genera had positive reactions. *P. ultimum* was detected by ELISA in roots of bean, cabbage, and sugar beet seedlings grown in pathogen infested soil. ELISA optical density readings for infected bean and sugar beet root samples were highly correlated ($r>0.9$) with infection levels determined by culturing the samples on water agar. The correlation between the two methods of testing cabbage roots was low, but all cabbage roots in which *P. ultimum* was detected by culturing were strongly positive in the ELISA. Samples of roots infected with *P. irregulare* and those with no *Pythium* infection did not react in the ELISA. The ELISA was highly sensitive; the fungus was detected in culture extracts diluted 1:5,000,000 and in roots with less than 1 infection per 100 cm root.

An antiserum was made against *Plasmodiophora brassiceae* the causal agent club of root of cabbage. A semipurified suspension of spores of *P. brassiceae* was used as antigen, obtained by filtration and percoll gradient contrifugation of infected roots. Crude root suspension samples were bound to a nitrocellulose nembrane 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. brassiceae,* 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. brassiccae* did not react with surface antigens of resting spores of *Polymyxa.* Further, no cross reaction with other common root pathogens such as *Pythium ultimum, Rhizoctonia solami,* and *Fusarium oxysporum* was observed. With antiserum prepared spore surface antigenic determinants the dot immunobinding technique can be used as routine test for detection of infection of *P. brassiceae* in host plant and in bait plants (used as indicators of soil infestation). The sensitivity obtained was within the range permisssible for a routine test (Lange *et. al.* 1989).

Two commercial serological assay Kits were compared to a culture plate method for detection of *Phytophthora cinnamomi* in root samples from inoculated azaleas (Benson, 1991). Both the multiwell E Kit and the rapid assay Kit detected *P. cinnamomi* on azalea roots beginning 1 wk after inoculation. Agreement between immunoassay Kits and culture plate results for detection of *P. cinnamomi* was most consistent beginning 3-5 week after inoculation. Root symptoms, but not foliar symptoms, of *Phytophthora* root rot were evident during this period. There was a positive correlation between root rot severity in greenhouse trials and root sample absorbance (multiwell) or meter reading (rapid assay) but not between symptom severity and immuno assay results. Although color reactions in the rapid assay detectors became increasingly darker after completion of the test, results after 5min were as reliable as those after 60 min since readings for uninoculated controls used to determine test thresholds also increased with time. The multiwell Kit detected *P. cinnamomi* in root samples containing as little as 1.0% infected root tissue. In a commercial nursery survey, 5 and 15% of the azalea root samples at two nurseries had positive ELISA

values that were unconfirmed by culture plate. The rapid assay Kit detected *P. cinnamomi,* was easy to use, and gave results in a short time.

Naphthazarin toxins of *Fusarium solani* were detected by Nemec *et. al.* (1991) using competitive ELISA analysis in xylem fluid of roots rotted by *F. solani* and symptomless scaffold roots and branches of healthy-apearing and diseased citrus trees in ridge and flatwoods Florida groves. Studies concentrated on blight, a wilt disease with an undefined cause and etiology, to determine if *F. soloni* is a causal factor of the disease. Healthy-appearing roots of trees with blight symptoms in six groves contained upto 11.4 times more toxin than roots of healthy trees in the some groves. In blight-diseased trees from these groves, mediam toxin values per root and the percentage of roots positive for toxin were higher than for healthy trees. Rotted roots from blight-diseased trees in two groves contained 112 and 3.4 times more toxin than healthy appearing roots from the diseased trees. In two groves, one containing tristeza-diseased trees and the other foot rot diseased trees, toxin concentrations were greater in diseased compared with healthy trees only in the foot rot site. Toxin concentrations were not different 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 roots infected by *F. solani,* immunocytochemical localization of naphthazarins was present in fungal cell walls and associated electron-dense substances on the outer surface of the hyphae. In the fungal cytoplasm, the toxin was localized in nonmembrane-bound electron-lucent areas. The presence of naphthazarin toxins in blight-diseased trees as well as those with other disease suggests the nonspecificity 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.

Field isolates of *Thielaviopsis basicola,* the causal agent of black root rot of cotton (*Gossypium hirsutum*), were grown in Czapek-Dox broth amended with dialyzed carrot extract. Soluble protein extracts of chlamydospores and mycelium were used to raise polyclonal mouse as cites antibodies. The immunoglubulin G antibody fraction was purified and biotin-labelled to devise a fungal capture sandwich enzyme-linked immunosorbent

assay (ELISA). ELISA detected both brown and grey cultural types of *T. basicola* and had negligible cross-reactivity with other soil brone fungi commonly found in the San Joaquin valley of California cotton field soils. The minimum detection limit of ELISA was between 1 and 20ng of *T. basicola* protein depending on the assay. *T. basicola* could be detected in cotton roots 2 days after inoculation. At this time, initial symptoms were apparent. The antibody also was used to observe *T. basicola* on cotton roots with immunofluorescence microscopy (Holtz *et. al.* 1994).

Purkayastha and Pradhan (1994) further studied serological differences among three strains (266, 23,M) of *Sclerotium rolfsi* along with their pathogenecities and antigenic relationship with five groundnut cultivars. Changes in antigenic patterns of one of its cvs. AK-12-24 and disease intensity were noted after treatment with a systematic fungicide Kitazin. Results reveal 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) of groundnut. The strain 23 also exhibited common antigenic relationship with cv. AK-12-24. Resistant cvs. JL-24 and ICGS-26, however, showed no antigenic relationship with fungal strains in either immunodiffusion or immunoelectrophoretic test. A systemic fungicide Kitazin EC 48% (500μg ml^{-1}) altered the antigenic pattern of roots of a susceptible cv. AK-12-24 and also reduced discase markedly.

Polyclonal antibodies were prepared against crude cell wall fractions of *Pythium* isolate with filamentous non-inflated sporangia, associated with root rot of tomato in soilless cultures. In ELIA tests, the antiserum showed sensitivity and specificity against original antigens and those from closely related isolates with filamentous sporangia (such as *Pythium aquatile* and *Pythium coloratum*). The antiserum reacted weakly with mycelial preparations of several fungi commonly found on tomato roots such as *Fusarium* spp. or *Penicillium* spp. An immunocytochemical staining test was developed in order to study the colonization by filamentous noninflated sporangial isolates of *Pythium* in the presence of other fungi in soilless cultures. (Rafin *et. al.*, 1994).

Pathogencity test of *Fusarium oxysporum* on ten cultivars of soyabean revealed soymax and Punjab-1 to be most resistant while JS-2 and UPSM-19 were most susceptible

(Chakraborty *et. al.* 1997). Antigens were prepared from the roots of all ten varieties of soybean and the mycelium of *F. oxysporum.* Polyclonal antisera were raised against mycelial suspension of *F. oxysporum* and the root antigen of the susceptible cultivar UPSM-19, cross reactive antigens shared by the host and the pathogen were detected first by immunodiffusion. The immunoglobulin fraction of the antiserum was purified by ammonium sulphate precipitation and DEAE-sephadex column chromatography. The immunoglubulin fractions were used for detection of cross reactive antigens by enzyme linked immunosorbent assay. In enzyme-linked immunosorbent assay, antigens of susceptible cultivars showed higher absorbance 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 fluorescein isothiocyanate indicated that in cross-sections 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 and chlamydospores of the fungus.

Srivastava and Arora (1997) detected and quantified *Macrophomina phaseolina,* causal agent of charcoal / dry root rot disease in many crop plants 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* f. sp. *ciceri.* Mycelial antigens of *M. phaseolina* on chickpea roots were detectable with DAS-ELISA at a minimum concentration of 10ng 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 patential antagonist (*Trichoderma harzianum* 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. phaseoline* under different environmental conditions.

Polyclonal antibodies (PAbs) were raised in white male rabbits immunizing separately with mycelia and cell wall antigens prepared from ten day old culture of *Ustulina zonata*

and emulsified with complete / incomplete adjuvants (Chakraborty *et. al.* 2001). Five blood samples were collected by marginal ear vein puncture from rabbits and IgG were purified DEAE-cellulose. The effectiveness of antigen preparations for raising Pabs were checked following immunodiffision 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 species 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. The authors suggested that such immunological methods developed for early detection of *Ustulina zonata* from soil and tea root tissue will be useful for the proper management of this disease.

B. Root and Leaf

Alba *et. al.* (1983) detected common antigens in extracts of urediniospores of *Hemileia vestatrix* and in leaf and root extracts of coffee plant prepared by different methods. An antigenic disparity was observed between coffee plants of physiologic groups D and E. The occurrrence of common antigens in coffee plants and urediniospores of *H. vestatrix* and their possible involvement in such interaction are diseased.

A commercial *Rhizoctonia* specific ELISA was compared with isolation on antibiotic amended water agar for the detection of *Rhizoctonia solani* and other *Rhizoctonia* spp. in asymptomic creeping bent grass near active brown patch disease centres. A greater number of positive samples were indicated by ELISA than by isolation in tests of foliage and root tissues. Over 75% agreement was found between ELISA and isolation with foliage, but the presence of *Rhizoctonia* spp. in root tissues could not be confirmed by isolation from any of the ELISA positive samples. There was poor agreement between the two methods in assays of organic matter from thatch. Greater numbers of thatch samples were positive for *Rhizoctonia* spp. by isolation than by ELISA. Pathogenic *R. solani* was found by both methods in asymptomatic foliage as far as 30 cm from the margins of brown

patch disease areas. There was a more restricted distribution of the fungus in the thatch. Detection of the fungus in root tissues from asymptomatic areas by ELISA could not be confirmed by isolation (Yuen *et. al.* 1984).

C. Stem tissue

Aguelon and Dunez (1984) developed an immuno enzymatic technique for the detection of *Phoma exigua* in infected potato tissues. Antisera to *Phoma exigua* var. *foveata* and var. *exigua* were prepared by injecting rabbits and mice with protein solutions from mycelium. Specific antibodies were isolated and immunoenzymatic techniques (double antibody sandwich ELISA and indirect ELISA) were used to test for the fungus in inoculated tubers and sports and in stems grown from these tubers. The fungus was detected in these different tissues, with var. *foveata* being more aggressive, demonstrating the applicability and sensitivity of the techniques. The antibodies produced to the two varieties of the fungus were not sepcific to their own varieties. They also reacted with *Phoma tracheiphila* but did not react with several other common potato pathogens. Preliminary results obtained with antibodies from mouse as cite liquid suggest the possibility of producing specific monoclonal antibodies.

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 wall fractions give 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. oxysporium* 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.

D. Leaf tissues

Purkayastha and Ghosal (1985) analysed the cross-reactive antigns of *Acrocylindrium oryzae* and rice in relation to sheath rot disease. Immunodiffusion, immunoelectrophoretic and crossed immunoelectrophoretic analysis of rice antigens in relation to sheath rot disease revealed a serological relationship between susceptible rice cultvars and isolates of the causal organism of sheath rot, *Acrocylindrium oryzae.* One precipitin band was observed when the antigen preparation of *A. oryzae* was cross-reacted with its own antiserum or against the antisera of four susceptible rice cultivars. No precipitin band was detected between the antiserum of the resistant cv. Mashuri and antigen preparations from three isolates of *A. oryzae* or between the antigens of the resistance cultivars Mashuri and Rupsail and the antiserum of *A. oryzae.* Crossed-immunoelectrophoretic tests confirmed that three was a common antigen between Mashuri and Jaya, and between Mashuri and CR-126-42-1. The precipitin band between the antigen preparation of Jaya and *A. oryzae* was found to be similar.

Sections of leaves of *Nicotiana tabacum* L. infected with *Peronospora hyoscymi* De Bary f. sp. *tabacina* (Adam) Skalicky and *Erythronium americanum* Ker. infected with *Ustilago heufleri* Fuekel were trreated 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 complex 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 indication the presence of fimbrial antigens, where as the control preparations showed only a low background level of labelling. Gold particles were detected on the section of hyphae, on haustoria, and on the nearby plant cells. The intensity of labelling was much higher for *P. hyocyami* f.sp. *tabacina* than for *U. heufleri* and was particularly high in the walls of the former species. Relatively high levels of labelling occurred over the cells of infected hosts, but little or none occured over the cells of uninfected host tissues, or of the infected host tissue treated with a range of serological controls. This high level of labelling was not associated with specific host structures in *P. hyoscyami,* but was frequently associated with the chloroplasts in *U. heufleri.* The antigens detected inside the host plant cells appear to indicate that fungal

fimbriae protein, either as polymerized fibrils 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 suggested the possibility that fungal fimbriae may play an important role in the molecular interaction between pathogen and host.

Though lesion formation by spore of *Botrytis cinerea* on *Vicia faba* occurred within 15h after inoculation, Gardiner *et. al.* (1989) reported that simultaneous application of various antisera delayed formation until up to 65h after inoculation. As the dilution of antiserum was increased, the length of the inhibitory period decreased and the fraction of the leaf surface that eventually became covered in lesion increased. Antisera of different antigen-binding specificity or different class of heavy chain (IgA, IgG, IgM) inhibited lesion formation, though IgM was the most effective. Antisera purified from other serum proteins were effective, but serum proteins such as albumins were not effective in causing inhibition of lesion formation. $F(ab^1)_2$ fragments of IgG molecules were as effective as the complete IgG molecule while Fc and light chain fragments were ineffective. Germination of *B. cinerea* spores on glass slides was inhibited by the same range of immunoglubulin molecules and fragments. The authors concluded that some constant portion of the antigen binding region of immunoglubulins inhibits lesion formation by inhibiting spore germination.

Ghosh and Purkayastha (1990) analysed the host parasite cross-reactive antigens in relation to *Myrothecium* infection of soybeam. For this purpose, antigens were extracted 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. Results of immunodiffusion revealed that common antigens were present only between the virulent strain and susceptible host cultivars. But no cross reactive antigen was detected in case of resistant cultivars (UPSM-19 and DS-73-16). Immunoelectrophoretic analysis showed that one common antigen was shared by susceptible hosts and the virulent strain (M-1). This was further confirmed by both crossed and rocket-immunoelectrophoresis.

A polyclonal antiserum prepared in a rabbit immunized with a mycelium extract of *Phytophathora 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. *P. infestans* mycelium in leaf tissue was readily detected by ELISA using either the plate-trapped antigen or F $(ab')_2$ antibody-fragment techniques. The amount of mycelium in leaf extracts was estimated by comparing the values obtained in ELISA with those for known concentrations of *P. infestans* mycelium (Harrison *et. al.* 1990).

In a further study Purkayastha & Banerjee (1990) used six antibiotics as foliar spray on a susceptible soybean cultivar (Soymax) to induce resistance against anthracnose. In addition, common antigenic relationship between seven soybean cultivars, their pathogens and non-pathogen were also studied using immunodiffusion, immunoelectrophoresis and indirect ELISA technique. Among the six antibiotics tested, cloxacillin and Penicillin induced maximum resistance against anthraenose. 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 *Colletotrichum 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 two at a very low level.

An immunocytochemical stain was developed 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. bombabacina,* incubated outside from movement 1986 to may 1987, and sampled monthly. Sections stained immunocytochemically showed that *A. bombacina* grew endophytically and epiphytically. The antagonist prevented neither growth of hyphae of *V. inaequalis* into the interior of leaves, nor initiation of psuedothecia. There was no particular spatial association between hyphae of the two

fungi, nor any sign of direct parasitism of hyphae or pseudothecia of *V. inaequalis.* Pseudothecia in leaves with the antagonist did not mature further than the stage of producing pseudoparaphyses, reaching an average of 84 μm in length. Pscudothecia in leaves without *A. bomabacina* developed asci normally and were 108μm long by 1may. These data were confirmed by results from an abbreviated sampling scheme of McIntosh apple leaves during 1987-1988 and by observations of pscudothecial inhibition in crab apple leaves following delayed application of the antagonist (Young and Andrews, 1990).

Ribosomal protein differences among snow mold fungi and wheat were utilized to detect the causal fungi in infected wheat leaves by indirect enzyme linked immunosorbent assay (ELISA) and western blot analysis (Takenaka, 1992). Polyclonal antisera were raised against the ribosomes of *Pythium paddicum, 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 antiserums, ribosomes of *P. paddicum* and *P. iwayamai* were serologically identical; the ribosomes of *T. inacarnata* and *T. ishikariensis* had partial common antigenic determinants, but there were apperent serological differences among *Pythium* spp. *Typhula* spp., *M. nivale* and wheat. With indirect ELISA, ribosomes of *Pythium* spp. were detectable at a concentration of 69 ng/ml and ribosomes of *T. incarnata* and *M. nivale* at a concentration of 210 ng/ml. The ribosomes of each target pathogen were detected from completely rotted wheat leaf homogenates diluted up to 1:1,000 or 1:10,000. The indirect ELISA could not differentiate, *P. paddicum* from *P. iwayamai*-infected plants or *T. incarnata* from *T. ishikariensis*-infected plants but could detect and differentiate snow mold fungi at the genus level in wheat plants. Western blot analysis with these antisera also could not differentiate *P. paddicam* from *P. iwayamai* but could differentiate wheat leaves infected with *T. incarnata* from those infected with *T. ishikariensis.* These immunological methods with antiribosome serums would be useful to evaluate wheat plants for infection by *Pythium* spp. *T. incarnata, T. ishikariensis* and *M. nivale.*

A new monoclonal antibody (MAb) was raised against haustorial complexes isolated from pea leaves infected with the powdery mildew fungus *Erysiphe pisi* by Mackie *et. al.* (1993). The antibody disignated UB10 recognises a 45 kDa N-linked glycoprotein which

is specially located in the haustorial plasmamembrane. This glycoprotein is clearly distinct from a previously characterized 62 kDa glycoprotein (identified with MAb UBB) which is alos specially located in the houstorial plasma membrane. These antibodies were used, along with MAb UB7 which binds to a major 62kDa glycoprotein in the cell wall plasma membrane of both haustoria and surface hyphae, to label haustoria with in epidermal strips from infected pea leaves using indirect immunofluorescence. Different stages of haustorial development can be observed using this procedure and the results showed that all three glycoproteins recognised by the MAbs are expressed early in haustorial development.

Two new monoclonal antibodies (MAbs) were further described which recognise glycoprotein components of the extrahaustorial membrane (ehm) which surrounds the haustorium formed during the infection of pea leaves by the powdery mildew fungus *Erysiphe pisi* (Roberts *et. al.* 1993). One of the MAbs, UB9, raised against enriched pea leaf plasma membranes, recognises a large (200kDa) glycoprotein in this membrane with in infected and uninfected epidermal cells. It does not label haustoria at early stages of development showing that at least one plasma membrane glycoprotein is excluded from the elm. However, UB9 binds to the elm of a subpopulation (approx 20%) of HCs late in the infection process and it is posible that the insertion of this glycoprotein into the ehm may represent a breakdown of biotrophy during senescence of haustoria or could be a late defence response by plant. A second MAb, UB11, was raised against isolates haustorial complexes derived from the pea powdery mildew system. This antibody recognises a glycoprotein (250kDa) which is present in the ehm of the haustorial complex and is expressed very early in haustorial development. UB11 does not bind to plant membranes of infected or uninfected plants and doesnot cross-react with other fungal components. This antibody therefore indentifies a glycoprotein specifically located to the elm of haustorial complexes and shows that there is molecular differentiation of this key interface between the biotrophic partners.

Among the Tocklai varieties of tea tested against *Bipolaris carbonum,* TV-18 was found to be most susceptible and TV-26 most resistant under identical conditions. Antigens obtained from tea varieties, isolates of *B. carbonum* and nonpathogens of tea (*Bipolaris tetramera* and *Bipolaris setariae*), were compared by immunodiffusion,

immunoelectrophoriesis and enzyme-linked immunosorbent assay to detect the cross-reative 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), nonpathogens and tea varieties, as well as nonpathogens and isolates of *B. carbonum.* Indirect staining of antibodies using fluoresein isothiociyanate (FITC) indicated that in cross sections of leaves (TV-18), the CRA were concentrated mainly around epidermal cells. Treatment of mycelia and condia 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 condia (Chakraborty and Saha, 1994).

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

Polyclonal antiserum was raised against the mycelial extract of *Pestalotiopsis theae* and immunoglobulin fractions were purified by ammonium sulfate fractionation and chromatography on DEAE - Sephadex. In enzyme linked immunosorbent assay, antiserum dilution up to 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 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 1:125, the pathogen could be detected on inoculated leaf extracts upto antigen concentration of 2mg/L. The antiserum reacted with two other isolates of *P. thae* tested but not with the antigens from mycelial extracts of *Glomerella cingulata* and *Corticium invisum* or with extracts of tea leaves inoculated with pathogen. The results demonstrated that ELISA can be used for early detection of *P. theae* in leaf tissues even at a very low level of infection (Chakraborty *et. al.* 1996a).

Varietal screening of tea (*Camellia sinensis*) with *Glomerella cingulata,* causal agent of brown blight disease was conductd with sixteen varieties released by Tocklai Experimental station, Jorhat, Assam, following detached leaf, cut shoot and whole plant inoculation techniques (Chakraborty *et. al.* 1996b). Among the tested varieties, TV-18 and Teen Ali-17/1/54 were found highly susceptible, while CP-1 and TV-26 were resistant under identical conditions. Plant antigens were prepared from healthy leaves of five selected varieties and fungal antigens were prepared from three isolates of *G. cingulata* (GC-1, 2&3) as well as non - pathogens of tea (*Bipolaris tetramera* and *Fusarium graminearum*). Rabbit antisera were raised against leaf antigens of TV-18, Teen Ali 17/1/54 and CP-1 and fungal antigens of *G. cingulata* (isolate GC-1). These antisera were used in immunodiffusion and immunoelectrophoretic tests as well as in enzyme linked immunosorbent assay (ELISA) to detect cross reactive antigen (CRA) shared between tea varieties and isolates 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 fluoresein isothiocyanate (FITC) indicated that in cross section of tea leaves (TV-18), the CRA an concentrated in epidermal cell, mesophyll tissue and vascular elements.

The initial infection process involving adhesion / recognition events between plants and fungal pathogens is essential for the establishment of pathogenesis (Laxalt *et. al.* 1998).

The basis of the specificity between *Phytophthora infestans* and potato is not yet known. It could lie in the recognition between extracellular matrix and plasma membrane components of the penetrating structures of the fungus and host cells during the earliest stages of infection process. Author used polyclonal antibodies (AZ and AC) obtained after immunizing rabbits with either 10^7 zoospores (Z) or geminating cysts (C) of *P. infestans* to detect putative fungal components located on the surface of invasive structures. AZ recognized several bands, while AC recognized three polypeptides of approximately 87, 73 and 45 kDa in western blots. Zoospore suspensions preincubated either with AZ or AC were unable to infect potato leaves. Since it was already known that some anti-idiotypic (anti-IT) antibodies may resumble the antigen, thus mimicking its structure, anti-IT antibodies (a-AZ and a-Ac) were generated in mice and used as inducers on potato tuber discs. In northern blots a-AZ treatments increased 1.5-fold the glyceraldehyde-3-P dehydrogenase (GAPDH) and 1.8 fold the phenylalanine ammonia-lyase (PAL) transcript levels, while an increase of 1.8 and1.7 fold for GAPDH and PAL, respectively, was observed when the inducer was a-AC. Both PAL and GAPDH transcript levels have previously been shown to be modulated by fungal and elicitor treatments. These results show that the anti-idiotypic antibodies are able to mimic the pathogen components, leading to the accumulation of transcripts related to defence responses in potato *P. infestans* interactions.

Pathogenecity of *Glomerella cingulata* (Stoneman) Spauld and Schrenk, causal agent of brown blight disease, tested 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-17/1/54 were susceptible. Polyclonal antisera were raised separately against antigen preparations from mycelia and cell wall of *G. cingulata* (isolate GC-1), 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* (GC-1, 2 and 3). Such antigens were not detected between raistant varieties of tea and isolates of *G. cingulata* (GC-1, GC-2 & GC-3); non-pathogen (*Fusarium oxysporum)* and tea varieties; isolates of *G. cingulata* and *F. oxysporum* and between non-host (*Glycine max, Cicer arientinum,* and *Camellia japonicum*) and *G. cingulata.* Antisera raised against cell

wall preparations gave better recognition from that against mycelial preparations as observed in ELISA test with antigens of tea leaves of different ages. (Chakraborty *et. al.* 2002).

E. Fruit

Antibody 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-antigody 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) identified immunochemically *Fusarium moniliforme* ribosomes from diseased corn (*Zea mays* L.) stalk tissue.

F. Seed

Distribution of the endophytic fungus, *Acremonium coenophialum,* with in the tissues of infected tall fescue (*Festuca arundinacea*) can be determined with tissue print-immunoblot (TPIB), however, the accuracy of this method in the determination of endophytic infestation levels of pastures and seed lots has not been evaluated (Gwinn *et. al.* 1991). The purpose of this study was to compare TPIB to other methods used for endophytic detection. Seed lots 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 pastures. Therefore, accuracy of TPIB in comparable to other endophyte detection techniques and can be used for routine detection of endophyte in tall fescue tissues.

Immunodetection of teliospores of Karnal bunt (*Telletia indica*) of wheat using fluorescent staining Test was done by Gupta *et. al.* (2000). Polyclonal antibodies were raised against intact teliospores in Newzeland white rabbits for the immunodetection of Karnal bunt (KB) teliospores. The indirect immunofluorescence (IIF) test was developed using anti-teliosperes serum and binding was monitored by goat-rabbit antibody conjugated to fluorescein (FITC) label. The standarization of IIF test was carried out by optimization of dilutions of anti-teliospores antibodies, fluorescent probe and exposure time. The

teliospores of *T. indica* showed bright green, patchy and ring shaped fluorescence around the teliospore. The spore exhibited uniform distribution indiscrete regions of spore probably in spore episporium. Similar fluorescence pattern in the teliospores of KB isolated from infected wheat seeds of cultivars HD 2328, UP 2338, PBW 393, WH 542, as well as RR 21 (susceptible cultivars) respectively, is an indication of the presence of similar antigenic configuration of teliospores. Again, they did not exhibit variation in the expression of teliospore associated molecular pattern (TAMP) during previous and subsequent years of infection.

Polyclonal antiserum were produced in albine white rabbits against intact teliospores. The immunoprove was used for the development of Immunoblot binding assay for detective infections in wheat seed samples. The antiserum reacted strongly with intact teliospores of *T. indica,* pantnagar isolate in agglutination reaction. The wheat grains with different grades of infection could be readily detected by Seed Immunoblot Binding Assay (SIBA). The teliospores of Karnal bunt infected wheat seeds when kept for vigour testing on nitrocellulose paper, formed a coloured imprint after the paper was assayed. The SIBA developed should not only be a better indication of teliospores load on seed but also quality of seed in terms of vigour. The developed immunodetection method appearently proves to be usefull in routine monitoring of wheat lots for the presence of K.B. pathogen (Kumar *et. al.*, 2000).

G. Whole plant

Methods for sampling turf grass tissue were compared by Shane (1991) for their effectiveness in monitoring Pythium blight epidemics with enzyme linked immunosorbent assay (ELISA). Sample areas consisted of marked strips on golf course fairways and tees with bent grass and annual blue grass naturally infested by *Pythium aphanidermatum.* Samples consisted of (1) whole plants picked by hand and assayed as whole plants; (2) whole plant sectioned into lower, middle, 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 very little change in ELISA reading for the

two lower strata. Several episodes of *Pythium* antigen increase were detected by ELISA assays of mowed samples, although signs and symptoms of Pythium blight were not evident. However, increase in ELISA reading for *Pythium* coincided with, but did not generally precede, the on set of blight symptoms with a 2-to 3-days sampling interval. Antibody-aided detection in useful for verification of diagnosis and determination of general *Pythium* population fluctuations, but current methodology in not satisfactory for advanced detection of blight spidemics.

H. Soil

MacDonald & Duniway (1979) used the fluorescent antibody to study the survival of *Phytophthora megasperma* and *P. cinnamomi* zoospore in soil. Zoospore cysts of *Pythophthora megasperma* and *P. cinnamomi* were adjusted to nonsterile soil and its matric potential was adjusted to predetermined values. The infested soils were held under laboratory and field conditions and periodically sampled to determined cyst viability and soil water potential. During sampling, soil containing cysts 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, zoospore cysts of *P. megasperma* survived no longer than 3wk at water ranging from 0 to –15 bars, and under field conditions they survived no longer than 2wk. Zoospore cysts of *P. cinnamomi* survived no more than 3wk 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, zoospore cysts of *P. cinnamomi* survived upto to 6wk in the laboratory. In a nonirrigated field plot, in which soil water potentiols ranged from –7 to less than –100 bars, cysts of *P. cinnamomi* survived for 8-10 wk. Under more controlled humidity conditions, zoospore cysts of *P. cinamoni* proved relatively tolerant of desication; many survived for 1wk in soil 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 1wk in soil dried to water potentials of –20 bars or less.

Polyclonal antisera were raised to whole (Coded :16/2), and sonicated (Coded : 15/2) resting spores of *Plasmodiophora brassicae,* and soluble components prepared by

filtration and ultracentrifugation (Coded : SF/2) (Wakeham & White, 1996). Cross-reactivity of all three antisera with range of soil fungi, including western blotting, dipstick, dot-blot, indirect enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence were assessed for their potential to detect resting spores of *P. brassicae* in soil. Dot-blot was least sensitive, with a limit of detection level of $1x10^7$ resting spores g^{-1} in soil. With western blotting the lower limit of detection with antiserum 15/12 was $1x10^5$. This antiserum showed the greatest sensitivity in a dip-stick assay, indirect ELISA and indirect immunoflurescence, for all of which there was a limit of detection of $1x10^2$. The indirect ELISA was successful only after the substitution of alkaline phosphatase by protein A conjugated horseradish peroxidase. Of the assays tested, indirect immunofluorescence appears to be the most rapid and amenable assay for the detection in soil of low levels of resting spores of *P. brassicae.*

It was reported by Walsh *et. al.* (1996) that a polyclonal antiserum produced against spore balls of *Spongospora subterranea* f. sp. *subterranea* prepared from potato tubers 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. How ever, the different spore ball contamination levels were discriminated better in ELISA tests at concentrations above 2000 spore balls per g soil than at lower concentrations. In contrast, a bioassay test based 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 inspired soils containing >2000 spore balls per soil. Tests on a limited number of field soils suggested, ELISA may be capable of predicting disease levels on tubers grown in such soil better than the biossay. The antiserum 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 eqully well. It also detected spores from different geographial origins. An attempt to improve the sensitivity of the serological detection through concentrating spore balls from field soils by sieving was unsuccessful. Cross-absorption of the antiserum with uncontaminated field soil increased the sensitivity of detection of spore balls in spiked soil samples four-fold. The ability of the antiserum to discriminate contaminated field soils from an uncontaminated soil was much improved by

using the gamma-globulin fraction or cross-absorbed screen. 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 autoflurescence of spore balls, allowing immunofluoresence studies to be carried out. Using this technique in conjunction with indirect FITC immunofluoreserence, discrete bright fluorescent spots were visualized using the specific serum. With the non-specific serum, only a very dull background fluorescence was evident.

Population of *Phytophtora* spp. were determined by Miller *et. al.* (1997) using enzyme-linked immunosorbent assay (ELISA) in field soils used for pepper and soybean production in Ohio Soybean fields were sampled extensively (64 fields, n = 6 samples per field over 2 years) and intensively (4 fields, n = 64 samples per field in 1 year) to assess heterogenecity of *P. sojae* populations. Four pepper fields (n = 64), three of which had a history of *Phytophthora* blight caused by *P. capsici*, also were sampled intensively during a 6-month period. Mean (m), variance (v), and measures of aggregation (e.g., variance-to-mean ratio, v/m) of immunoassay values, translated to *Phytophthora* antigens units (PAU), were related to the disease history in each of the pepper and soybean fields. Mean PAU values for fields in which *Phytophthora* root rot (soybean) or blight pepper had been moderate to severe were higher than in fields in which disease incidence had been low or not observed. A detection threshold value of 11.3 PAU was calculated with values for 64 samples from one pepper field. All of which tested negetive for *Phytophthora* by bioassay and ELISA. Seven of the eight intensively sampled fields contained at least detectable *Phytophthora* propagules, with the percentage of positive samples ranging from 1.6 to 73.4. Mean PAU values ranged from 1 to 84 (extensive soybean field sampling), 6 to 24 (intensive soybean field sampling), and 4 to 30 (intensive pepper field sampling); however, various ranged from 0 to 7,744 (extensive sampling), 30 to 848 (intensive soybean field sampling) and 5 to 2, 401 (intensive pepper field sampling). Heterogenecity of PAU was high in most individual soybean and pepper fields, with values of v/m greater than 1, and log (v) increasing with log (m), with a slope of about 2.0. Spatial autocorrelation coefficients were not significant, indicating there was no relationship of PAU values in neighbouring sampling units (i.e., field locations) of the intensively sampled fields. Combined results for autocorrelations and v/m values indicate that *Phytophthora* was highly

aggregated in these fields but that the scale of the aggregation (e.g., average focus size) was less than the size of the sampling units. Because of the observed variability, authors calculated that sample sizes of 20 or more would be needed to estimate precisely the mean density of *Phytophthora* in most causes.

Soil borne disease caused by such pathogens as *Fusarium oxysporum* and *Plasmodiophora brassicae* are often difficult to be controlled by using soil fumigants and / or conventional fungicides (Aric-Tsutomu 1998). This is mostly due to the survival powers of the pathogens in soil and the mode of infection through the plant root system. For integrated pest management (IPM) method, researchers recently recommended the combined application of agrochemicals, resistant cultivars, biocontrol agents, and soil amendment, to guarantee plant health. Although importance of intial diagnostics for soilborne pathogens in field soil and young nursery seedlings should not be ignored to build an IPM program and obtain the expected results, no practical diagnostic methods, have been reported other than the isolation of the pathogens on selective media. Current immunology provides rapid, specific and sensitive tools for the detection of pathogens. In fact, various immunological procedures have been commonly applied to detect human pathogens and plant viruses. With some modifications / improvements, these procedures can be used to diagnose plant disease, caused by various pathogens.

I. Organism

The species of the genus *Phytophthora* was differentiated serologically by the means of gel diffusion and immunofluorescence (Burrell *et. al.* 1965). 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 hyphae. Species-specific sera were obtained and proved useful and efficient for the identification of *P. cactorum, P. cinnamomi,* and *P. erythrosepticca.*

Using the serological techniques of agglutination, gel diffusion, and immuno fluorescence, identification studies were performed by Amos & Burrell (1966) on eight species within the genus *Ceratocystis : C. fagacearum, C. pluriannulata, C. variospora, C. radicicola, C. pilifera, 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 adsorb selectively cross-reacting antibodies from the sera to make them species-specific. The immunofluorescent technique proved to be the most useful in differentiating among the species. Although all of the species could be shown to have serological differences, no such differences could be seen between A and B compatibility types of *C. fagacearum.*

Merz *et. al.* (1968) further reported 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 was increased by the addition of ethylenediamine tetracetate 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. These methods were used to study the taxonomic relations of six heterothallic species of *Phytophthora. Phytophthora cinnamomi* was serologically distinct, whereas two serological groups were evident among five closely related species when tested with antiserum to *P. arecae. Parecae, 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.*

Immunological comparison of three strains of *Fusarium vasinfectum,* differing in their pathogenicity and a strain of *F. cubense* revealed close identity between pathogenic strains of *F. vasinfecum* (Kalyansundaram & Charudattan 1969). The three strains of *F. vasinfectum* had however, certain major antigenic constituents in common with *F. cubense.* According to Charudattan (1970) the pathogenic and non-pathogenic strains of *F. vasinfectum* can be differentiated by the production of the wilt toxin, fusaric acid. Higher toxin production was seen in a medium with nitrate as the source of nitrogen than with ammonical or organic sources of nitrogen. With peptone, there was maximum growth but minimum of toxin synthesis. No strict correlation was noted between growth and toxin production. The non-pathogenic strain (p_1) produced uniformly higher quantity of toxin than the two pahogenic strains (P_1 and A_1). No strict correlation was noted between the chemical presence of the toxin and its biological activity. The American pathogenic strain

A_1 produced maximum pectin methylesterase and very little polygalacturonase while the reverse was tree with the pathogenic Indian strain P_2. Perhaps, this quantitative difference accounts for the specificity of the two strains to produce disease in their respective hosts. Using agar double diffusion technique, a comparison of the antigenic components of the strain revealed a very close antigenic relationship between the two pathogenic strains P_2 and A_1 obtained from two different countries geographically much separated. Although, the pathogenic strains P_2 and A_1 possessed many antigens in common with those of non-pathogenic strain P_1, they both had a specific antigen which was lacking in P_1. However, the host specificity of the 2 pathogenic strains could not be explained by those findings. Non-pathogenic strains (P_1) not only lacked the above common antigen of P_2 and A_1, but also had its own specific antigen. Since this strain was pathogenic on original isolation, the present protein pattern might have evolved due to mutation arising out of prolonged maintenance in cultures.

A strain-specific antiserum was prepared by Horrok & 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 homologous *F. culmorum* strain and those of the heterologous *F. graminearum* and *F. culmorum* strains. All the other *Fusarium* species tested showed no detectable fluorescence. The heterologous strains of *F. culmorum* and *F. graminearum* could not be distinguished. No significant difference was found between the "culmorum" strains from different host plants. The serum specific for *F. culmorum* and *F. graminearum* is suitable to separate these species from other fungi. According to the the authors morphological examination and culturing processes are not necessary because mycelia sufficient for fluorescent antibody staining usually grow out from infected plant on to a microscope slide within 48-72 hours.

The serological cross-reactivity of *Sporothrix schenckii* with vaious unrelated fungi was investigated by use of immunodiffusion tests. A rabbit anti *S. schenckii* serum was obtained, which reacted with *Cladosporium wereneckii, C. carrionii, C. bantianum, Coccidioides immitis, Phialophora jeanselmei, P. gougerotii, P. dermatitidis,*

Fonsecaea pedrosoi, Aspergillus fumigatus, Histoplasma capsulatum and *Trichophyton mentagrophytes,* but not with *Saccharomyces cerevisiae* antigens. The serological determinants responsible for the cross-reactions were suggested by Ishizaki *et. al.* (1981) to be D-galactosyl residues.

Four *formae speciales* of *F. oxysporum* (*dianthi, melonis, pisi, lycopersici*) and three physiological races (1, 2, 3) of *F. oxysporum* f. sp. *melonis* have been differentiated using specific antisera and the 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 appropiate antisera reacted the same within the each race. (Ianelli *et. al.* 1982).

Nachmlas *et. al.* (1982) isolated a phytotoxin protein-lipopolysaccharide (PLP) complexe from dialysed culture fluids of a pathogenic strain of *Verticillium dahliae;* PLP complexes which lack phytotoxic activity were isolated from a mutant non-pathogenic strain of the fungus. A comparison of profiles of the wild-type and mutant PLP eluting from an Agarose A-5m Column revealed quantitative differences between the 2 major protein peaks of each strain. The corresponding peaks of the 2 strains had similar molecular weights and gel imunodiffusion 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 extract of *Verticillium*-infected potato plant tissue which was apparently indentical to a moiety produced by the pathogen in culture.

Cross-reactive antigens were detected by Alba & Devay (1985) in crude preparations and in purified preparations from mycelia of *Phytophthora infestans* Race 4. and Race 1.2. 3.4.7. with antisera for potatoes cv. King Edward and cv. Pentland Dell by using an indirect ELISA technique. 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 concentrated 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. mycelial preparation was assayed with antisera for King Edward and Pentland Dell.

The adaptation of enzyme-linked immunosorbent assay for the detection of *Sclerotiania 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}.

Cross-reactivity between antisera produced against fimbriae of either *Ustilago violacea* (Pers.) (AU) or of *Rhodotorula rubra* (Demme) Lodder (AR) and cell surface proteins of two ascomycete fungi, *Ascocalyx abietina* (Lagerberg.) Schlaepfer-Bernhard and *Ophiostoma ulmi (Buism)* Nannf., was revealed by dot-immunobinding and immunocytochemical methods. 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 labeling intensity was always found to be greater over the plasma membrane. The fibrillar sheath surrounding cells of *A. abietina* reacted with antiserum AU while all other structures did not. No significant labeling with this antiserum occurred over cells of *O. ulmi* indicating that they either lacked these antigens or that they were more easily removed during the fixation process (Benhamou *et. al.* 1986).

The agglutination effect of five lectins having different sugar-binding specifities on the conidia of a variety of strains from eight *Fusarium* species was examined by Cristinzio *et. al.* (1988). Conidia of all the strains of *F. solani* and *F. oxysporum* were strongly agglutinated by concanavalin A and *Helix pomatia* agglutinin; D-mannose (and D-glucose) and N-accyl-D-galactosamine inhibited the agglutination induced by concavalin A and *Helix pomatia* agglutinin, respectively. Condia of the other species of *Fusarium* examined (*F. culmorum, F. sambucinum, F. graminearum, F. avenaeum, F. moniliforme* and *F. xylarioides*) were not agglutinated by either lectin. No conidia form the *Fusarium* species were agglutinated by wheat-germ agglutinin, potato lectin and *Ulex europaeus* agglutinin-I. These results have provided some insight into the chemical nature of the outermost layer of *Fusarium* conidia and this information may be of taxonomic significance.

Fuhrmann *et. al.* (1989) immunologically differentiated the *Penicillium* species. Anisera were obtained from a rabbit immunized with *Penicillium verrucosum* var. *verrucosum*. These antisera were characterized by immunofluorescence and by indirect

enzyme-linked immunosorbent assay for their reactivity with 44 strains of moulds. Antigenically, *P. verrucosum* var. *verrucosum* (subgenus *Penicillium)* appears to be similar to strains belonging to subgenus *Furcatum,* but strongly different from *Penicillium frequentans* (subgenus *Aspergilloides*). Specific absorption of antibodies to antigens confirmed the existence of similar biochemical structures on *Penicillium frequentans. Aspergillus versicolor,* and *Aspergillus fumigatus.*

Competitive types of two novel enzyme-linked immunosorbent assays (ELISA) for *Fusarium* species were developed. Antiserum against strain F 504 of *F. oxysporum* was elicited in rabbits, and a highly specific, sensitive and accurate ELISA for the homologous strain 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* F 501 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 (Kitagawa *et. al.* 1989).

Soluble proteins from 34 isolates representing 16 species of the genus *Phytophthora,* collected from different geographic locations and host plants, when analysed by two dimensional electrophoresis, were resolved into 100 to 200 protein spots with diferent densities. Protein patterns differed markedly among different species and each species exhibited a distinctive and characteristic pattern. Although slight differences were occasionally observed in patterns of faintly-stained minor protein spots, those of densely-stained major protein spots were highy similar, if not identical, among isolates of the same species regardless of their geographic origin, original host plant, mating type, or virulence race. These reslts suggested that the two-dimensional electrophoretic patterns are species-specific and may be useful measure to differentiate and identify species of genus *Phytophthora* (Masago *et. al.* 1989).

Glycoconjugates on the surface of zoospores and cysts of the pathogenic fungus *Phytophthora cinnamomi* have been studied by Hardham & Suzaki (1990) using

fluorescein isothocyanate labelled lectins for fluorescene microscopy and flow cytometry, and ferritin-and gold-labelled lectins for ultrastructural analysis. Of the five lectins used, only concanavalin A (ConA) binds to the surface of the zoospores, including the flagella and water expulsion vacuole. This suggested that of accessible saccharides, glucosyl or mannosyl residues predominate on the outer surface of the zoospore plasma membrane. Early in encystment, a system of flat disc-like cisternae which underlie the zoospore plasma membrane, vesiculate. These and other small peripheral vesicles quickly disappear. After the induction of encystment, ConA is no longer localised close to the plasma membrane but binds to material loosely associated on the cell surface. Quantitative measurement by flow cytometry indicate that the ConA binding material is gradually lost from the cell surface. The cyst wall is weakly labelled, but the site of germ tube emergence stain intensely. Dering the first 2min. after the induction of encystment, material that binds soybean agglutinin, *Helix pomatia* agglutinin, and peanut agglutinin appears on the surace of the fungal cells. The distribution of this material, rich in galactosyl or N-acetyl-D-galactosaminosyl residues, in initially patchy, but by 5 min the material evenly coats mostly of the cell surface. Labelling of zoospores is which intracellular sites are accessible indicates that the soybean agglutinn binding material is stored in vesicles that lie beneath the plasma membrane. Quantitation of soybean agglutinin labelling shows that maximum binding occurs 2-3min after the induction of encystment.

Harold *et. al.* (1990) isolated antibodies to three isolates each of *Armillaria mellea, A. ostoyae, A. tabescens,* and *Lentinula edodes* from eggs of immunized laying hens. The reactivity of each antibody preparation with the isolates was examined using an enyme-linked immunosorbent assay (ELISA). The cross reactivity of the antibody preparations to a given *Armillaria* species varied considerably when tested against antibodies of other *Armillaria* species. Several antibody preparations were capable of distinguishing isolates of a homologous species from isolates of heterologous species. The specificity of the antibodies present in eggs depended on time elapsed since immunization. Eggs laid 3 weeks after immunization with a *Armillaria* specis isolate possessed antibodies that were most specific for isolates of that species. The intergeneric cross reactivity was found to be smallest with antibodies from eggs laid 5 weeks or more after immunization.

Extracts from *Fusarium oxysporum* (F.O.) and *F. oxysporum* var. *redolens* (F.O.R) isolates were compared by means of electrophoresis and cross immunoelectrophoresis (Rataj-Gunanowska and Wolko 1991). The polymorphism of five isozyme systems allowed a distinction between *F. oxysporum* and *F. oxysporum* var. *redolens* isolates. The isozyme patterns of three other isozyme systems did not allow this distinction between *F. oxysporum* and *F. oxysporum* var.*redolens* to be made. Both fungi apeared 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. oxysporum* var. *redolens* isolates. Although the results obtained indicate a strong similarity between *F. oxyporum* and *F. oxysporum* var. *redolens* they are not sufficient for an unequivocal statement that the fungi belong to the same species.

Wycof & Ayer (1991) used a set of five carbohydrate-specific monoclonal antibodies (mAbs) to probe the ultrastructure of the walls of the soybean pathogen *Phytophthora megasperma* f.sp. *glycinea,* using a combination of immunofluorescence and immunogold labelling techniques. Results with β-1, 3 glucan-specific antibodies suggest that β-1,3 glucans are present throughout the walls of both germtubes and cysts, but are more prevalent in the outer portion. In addition, β-1,3 glucans on the surface of the hyphal walls, but not cysts, are closely associated with other material, most likely protein, that sterically hinders antibody binding except to non-reducing terminal residuces. 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 are present on the exterior of *P. megasperma* f. sp. *glycinea* walls as well. Staining with another antibody indicates that changes in wall composition occur over 50-100μm from the hyphal tip, a greater distance than previously supposed. The role of the antigens recognised by these mAbs in the plant pathogen interaction is not known, but potential uses of these and other mAbs are discussed.

Polyclonal antibodies (PAbs) were produced in New Zealand white rabbits with culture filtrate and mycelial extract immunogen preparations from the soybean (*Glycine max*) fungal pathogen *Phomopsis longicolla* (Brill *et. al.* 1994). The PAbs were purified to the immunoglobulin fraction and tested in indirect ezyme 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 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 wih 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 member of the complex at the some time. Reactivity to same cultures of *P. longicolla* was undetectable following the fourth and fifth immunizations, whereas reactivity of all of other cultures of the complex remain high.