Literature Review

In nature a large number of interactionsbetween microorganisms and plants take place but most of them do not result in the entry of the pathogen into the host. Succesful host pathogen interaction generally depends on the recognition of the pathogen by the host. It is generally accepted that the cells recognise one another through pairs of complimentary structures on their surfaces; a structure on one cell carries encoded biological information that the strucrture on the other cell can decipher. Thus disease develops in individual plants by a series of sequential steps begining with the arrival of the inoculum at the plant surface and ending with the terminal stages of pathogenesis (Cowling and Horsfall; 1978). These stages in between the first and the last are unique to each host and pathogen and also depends to some extent on the prevailing environmental conditions. Serological relationship between host and pathogen is one of the first determinants in the establishment of pathogen within the host. Consdering the importance of serological relationships for host pathogen interaction a short selective review along these lines pertaining to the present study has been presented in the following pages. The review has been divided into three parts: (a) serological cross selectivity between host and parasite (b) detection of plant pathogenic fungi and (c) serological differentiation amongst and fungal species.

Serological cross reactivity between host and parasite

It is now well established that an immune system functionally similar to that of animal exist in plants. The serological cross reactivity between host and pathogen has been a subject of considerable interest to a number of workers and a number of review pertaining to this area have been published previously (DeVay and Adler, 1976; Damien, 1964; Clark, 1981; Chakraborty, 1988; Purkayastha, 1989; Purkayastha *et.al* 1991 and Purkayastha, 1994).

Serological methods for the differentiation between resistant and susceptible varieties of cotton infected with *Fusarium oxysporum* and *Citrus* sp. with *Phytophthora citrophthora* have been described by Abd-El-Rehim and Hashem (1970) and Abd El Rehim *et al.,* (1971 a). Serological and immunoelectrophoretical investigation on water melon varieties, resistant and susceptible to *Fusarium semitectum* also revealed that the cultivars could be differentiated by the titre or the time after which reaction occurred between antisera specific to the pathogens and seed globulins. It was noted that a,b globulin was present only in the resistant varieties. (Abd-El-Rehim *et. al.*, 1971 b).

Wimalajeewa and DeVay (1971) detected common antigenic relationship between *Zea mays* and *Ustilago maydis*. A pair of compatible haploid lines and two diploid solopathogenic lines of *U. maydis* were used in serological studies. *Avena sativa* var.'Victory' and *Hordeum vulgare* var. 'California Mariout' were taken as resistant hosts.Certain antigens were found common between Corn and *U. maydis*. A strong antigenic relationship existed between the solopathogenic lines 132 and 3 day oat seedlings. Barley did not have any antigen in common with any of the *U.maydis* did not indicate any qualitative significant serological difference among them.

Charudattan and DeVay (1972) compared antigenic preparation from four varieties of *Gossypium hirsutum* and isolates of *Fusarium* and *Verticillium* species for the presence of common antigen. At least one antigenic substance was common between the varieties of cotton and isolates of *Fusarium oxysporum* f. sp. *vasifectum*, *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. moniliformae* (nonpathogenic) and cotton. In gel diffusion tests five to eight precipitation 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 did not appear to be related to the severity of wilt symptoms , but possibly affected host pathogen compatibility during the process of root infection. Cross reactive antigens were also found between eight legume hosts and three *Rhizobium* species (Charudattan and Hubbell, 1973). These common antigens among hosts and bacteria were not related to the specificity of compatible *Rhizobium* - legume association. The cross-reactive antigens were absent between rhizobia and eight non-legume plants tested, but present between five out of eleven gram-negative phytopathogenic bacteria and legumes.

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

Antisera raised against soluble extracts of (Race-4) and tubers of 'Arranbanner' and "Golden wonder" potato cultivars showing field susceptibility and resistance respectively

to late blight were used to test for the presence of common antigens between extracts of the fungus and various host and non host plants (Palmerley and Callow, 1978). Cross reactive antigen was detected between *P. infestans* (Race-4) and potato tubers of both the field susceptible and field resistant cultivars and also between the fungus and leaves of tomato and tobacco.Common antigens were not detected between *P.infestans* (Race-4) and leaves of non host (mungbean, pea, radish, cucumber and maize), or between potatoes and the alternative pathogen, (*Fusarium solani* var. *acruleum*) and two non pathogens (*Ustilago maydis* and *Phytophthora cinnamoni*)

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

Conidia of *F. oxysporum* f. sp. *vasinfectum* was reported to contain an antigen that cross-reacted with antiserum to cotton root tissue antigens (Charudattan and DeVay, 1981). In agar gel double diffusion tests, one precipitin band was formed when antiserum to cotton antigens was reacted with crude fungal antigens, or when antiserum to crude fungal antigens was reacted with cotton antigens. The cross reactive antigen from fungal conidia (CRA) was isolated, purified and partially characterized. The CRA migrated as a single band in polyacrylamide or agar gel electrophoresis, and sedimented as a single band during analytical ultracentrifugation. It was antigenic in rabbits, and was a protein carbohydrate complex. The major cross-reactive antigenic substance (CRA), common to cotton (*Gossypium hirsutum*) and certain fungal parasites of cotton roots, was further purified to homogeneity from conidial cultures of *F. oxysporum* f.sp. *vasinfectum* (DeVay *et al.*, 1981). Agar gel double diffusion tests indicated the presence of CRA not only in *F. oxysporum* f. sp. *vasinfectum* and in cotton roots and seed but also in *Thielaviopsis basicola*. Indirect staining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross-sections of roots, cut near or just below the root hair zone, the CRA was concentrated mainly around xylem elements, the endodermis and epidermis cells and was present throughout the cortex tissue. Protoplasts prepared from cross-sections of young cotton roots also contained the CRA which was concentrated in the region of the plasmalemma. Treatment of conidia and mycelia of *F. oxysporum*

f. sp. *vasinfectum* with antiserum to cotton and using indirect staining with FITC indicated that the CRA was mainly present in hyphal tips and in patch-like areas on conidia.

Chakraborty and Purkayastha (1983) detected cross reactive antigen shared between soybean cultivars and *Macrophomina phaseolina* causing charcoal rot disease. Rabbit antisera were raised against root antigens of soybean cultivars (Soymax and UPSM-19) and *M.phaseolina* isolate (M.P. 1) and tested against homologus and heterologous antigens following immunodiffusion test. When antiserum of *M. phaseolina* was reacted against its own antigens and antigens of susceptible soybean cultivars (Soymax, R-184) strong precipitation reactions were observed. In case of resistant cultivars (UPSM-19 and DS-73-16) no such reactions were observed. Reciprocal cross reactions between antiserum of the resistant cultivars and antigens of three isolates of *M. phaseolina* also failed to develop even weak precipitation bands. Four antigenic substances were found to be common between the susceptible soybean cultivars and isolates of *M. phaseolina* in immunoelectrophoretic tests, but no common antigens were detected between resistant cultivars and the fungus. Purkayastha and Chakraborty (1983) further detected that in susceptible soybean plants (cvs. Soymax and R-184) a close relationship exists between lower production of glyceollin and presence of common antigens. The production of glyceollin was much higher in resistant soybean cultivars (cvs. UPSM-19 and DS-73-16) where common antigens were absent. Alba *et al.*, (1983) also detected common antigens in extracts of *Hemileia vastatrix* urediniospores and of *Coffea arabica* leaves and roots.

Alba and DeVay (1985) detected cross-reactive antigens in crude preparations and in purified preparations from mycelia of *Phytophthora infestans* Race-4 and Race-1, 2,3,4,7 with antisera for potatoes cv. King Edward and cv. Pentland Dell by using an indirect ELISA technique. It was suggested that the fungal mycelia do not easily release cross-reactive antigens into synthetic media where they grow; that most of *P. infestans* cross reactive antigens were thermolabile and that they could be concentrated by precipitation in the presence of 40% saturated ammonium sulphate (SAS). An antigenic disparity was observed when 40% SAS from *P. infestans* Race-4, mycelial preparation was assayed with antisera for King Edward and Pentland Dell.

Antigenic determinants responsible for a precipitation line specific for *Mycena galopus* were partially purified using salt fraction (with ammonium sulphate) and

ion exchangechromatography (Chard *et al,* 1985). Fractions 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. Antigens extracted from four near isogenic barley lines when cross reacted with the antisera of *Erysiphe graminis* f.sp. *hordei*) were shown to share immunologically identical antigens (Heide and Swedegard Peterson, 1985).

Immunodiffusion, immunoelectrophotetic and crossed 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*. One precipitation band was observed when the antigen preparation of *A. oryzae* was cross-reacted with its own antiserum or against the antisera of four susceptible rice cultivars. No precipitin band was detected between the antiserum of the resistant cv. Mahsuri and antigen preparations from three isolates of *A. oryzae* or between the antigens of the resistant cultivars Mahsuri and Rupsail and the antiserum of *A. oryzae*. Crossed immunoelectrophoretic tests confirmed that there was a common antigen between Mahsuri and Jaya, and between Mahsuri and CR-126-42-1. The precipitin band between the antigen preparation of Jaya and *A. oryzae* was found to be similar (Purkayastha and Ghosal, 1985). The common antigenic relationship between soybean cultivars and *Colletotrichum dematium* var. *truncata* was also ascertained by Purkayastha and Banerjee (1986) following immunodiffusion, immunoelectrophoretic and crossed immunoelectrophoretic tests. At least one antigenwas found to be common between host cultivar and the pathogen. No antigenic relationship was observed either between soybean cultivars and the non pathogen (*C.corchori*) or avirulent pathogen (*C.dematium*).

Purkayastha and Ghosal (1987) also compared the antigenic preparations from two isolates of *Macrophomina phaseolina* , a pathogen of groundnut, four non-pathogens of groundnut (viz., *Corticium sasakii, Colletotrichum lindemuthianum, C. corchori,* and *Botrytis allii*) and five cultivars of *Arachis hypogea* by immunoserological techniques. Common antigens were found among the susceptible cultivars of groundnut and two isolates of *M.phaseolina,* but not between nonpathogens and groundnut cultivars. No antigenic similarity was found between nonpathogens and *M.phaseolina* isolates. Cross-immunoelectrophoretic tests confirmed that at least one antigen was common between cv. J-11 and cv. TMV-2, cv. Kadiri 71-

1 and cv. TMV-2 and cv.Kadiri 71-1 and isolates of *M.phaseolina.*

Chakraborty and Purkayastha (1987) reported the changes in antigenic patterns after chemical induction of resistance in susceptible soybean cultivar (Soymax) to *Macrophomina phaseolina.* Sodium azide (100 μg/ml) altered antigenic patterns in susceptible cultivar (Soymax) and reduced charcoal rot disease. Similar results were also obtained by Ghosal and Purkayastha (1987) in susceptible rice cultivar (Jaya) and *Sarocladium oryzae* after altering disease reaction (sheath rot) by the application of gibberellic acid and sodium azide (100 μg/ml).

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

Purkayastha and Pradhan (1994) observed that three strains of *Sclerotium rolfsii* were serologically different and their pathogenecities also differ markedly with host cultivars. Virulent strains 266 and 23 showed common antigenic relationship with their respective susceptible cost cultivars but not resistant cultivars. Antigenic change in a susceptible cv. PK-12-24 after treatment with a systemic fungicide kitazin was also evident. They suggested that the resistance could be induced in susceptible plants of specific antigens were

eliminated by suitable treatment.

Antigens obtained from six Tocklai varieties of tea, four isolates of *Bipolaris carbonum* and non-pathogens of tea (*Bipolaris tetramera* and *Bipolaris setarae*), were compared by immudiffusion, immunoelectrophoresis and indirect ELISA in order to detect cross-reactive antigens (CRA) shared by the host and the parasite. CRA were found among the susceptible varieties (TV- 9, 17 and 18) and isolates of *B.carbonum* (BC 1,2,3, and 4). Such antigens were not detected between isolates of *B.carbonum* and resistant varieties (TV-16, 25 and 26), non-pathogens and tea varieties, as well as non pathogens and isolates of *B.carbonum*. Indirect straining of antibodies using fluorescein isothiocyanate (FITC) indicated that in cross sections of leaves (TV-18), the CRA were concentrated mainly around epidermal cells. Treatment of mycelia and conidia of *B.carbonum* with antisera to leaves (TV-18) and indirect staining with FITC indicated the presence of CRA in the young growing hyphal tips and conidia (Chakraborty and Saha, 1994 b).

Among the 12 varieties of tea tested against three isolates of *Pestalotiopsis theae*, causal agent of grey blight disease, Teen Ali-17/1/54 and TV-23 were found to be highly susceptible while CP-1 and TV-26 were resistant under identical conditions. Leaf antigens were prepared from all the tea varieties, three isolates of *P.theae* and a non-pathogen of tea (*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 immudiffusion 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*.. 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* (Chakraborty, *et.al.*, 1995).

Detection of Plant Pathogenic Fungi

Detection of plant pathogenic fungi within host tissues by serological means is a relatively recent development in the field of plant pathology. The merit of this method of detection lies in its ability to detect even very small amounts of pathogen in tissues which is generally not detected by conventional techniques. A number of recent reviews have been published by workers along this line (Hansen and Wick, 1993 and Werres and Steffens, 1994)

Detection of *Ophiobolus graminis* on the roots of cereals and its rhizosphere by the use of direct and indirect fluorescent antibody staining was reported. *Aspergillus* and *Penicillium* spp. were the predominant fungi isolated in culture from 5 samples of stored grain. Using an immunofluorescence 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 amounts of *Alternaria* mycelium were detected in grains of all the samples (Warnock, 1973).

Casper and Mendgen (1979) used an ELISA format to estimate *Verticillium lecanii* at different stages of infection in leaves of wheat infected with yellow rust pathogen (*Puccinia striiformis*). Following immunofluorescence technique, *V.lacanii* could also be detected in pustules of rust pathogen (*Uromyces phaseoli*) of bean

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

Antibodies raised to the ribosomes of *Fusarium moniliforme* and corn (*Zea mays.*) 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 (Michael and James, 1981).

Johnson *et al.* (1982) used antiserum prepared to homogenates of washed *Epichloe typhina* mycelium grown in a liquid medium in an enzyme-linked immunosorbent assay (ELISA) to detect antigens of the fungus in tall fescue (*Festuca arundinacea*) tissue samples. Very low concentrations (100 µg/ml) of freeze-dried *E.typhina* mycelium could be detected and the pathogen could also be detected in individual seeds of tall fescue. Of 14 fungal genera tested, including *Acremonium, Claviceps, Helminthosporium, Pythium, Rhizoctonia* and *Sclerotium* all showed reactivities less than 0.1% that of *E. typhina.*

Antisera were made to both a whole cell and cell wall preparation of *Eutypa armeniacae*. Rhodamine isothiocynate (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 viticola*. Woody cross sections from concord grapevines inoculated with *E.armeniacae* and also inhabited by various other fungi were stained directly with the conjugated anti-*Autypa* rabbit serum. In an indirect staining procedure, sections were treated with anti-*Eutypa* rabbit γ globulin. Both procedures specifically stained hyphae in wood sections. Hyphae stained indirectly in woody vine sections showed a much brighter fluorescence than analogous hyphae stained by the direct method. Fungi of some species that reacted strongly with the direct method on glass slides did not always react when stained indirectly in woody vine sections in which they were growing (Gendloff *et al.*, 1983).

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

Aguelon and Dunez (1984) used double antibody sandwich ELISA and indirect ELISA technique for the detection of *Phoma exigua* in inoculated tubers and sprouts and in stems grown from these tubers. The fungus was detected in these different tissues with var. *foveata* being more aggressive, demonstrating the applicability and sensitivity of the techniques. The antibodies produced to the two varieties of the fungus were not specific to their own varieties. They also reacted with *Phoma tracheiphila* but did not react with several other common potato pathogens. Preliminary results obtained with antibodies from mouse ascite liquid suggest the possibility of producing specific monoclonal antibodies.

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

Sections of leaves of *Nicotiana tabecum* infected with *Peronospora hyoscyami* f.sp. *tabacina* and of *Erythronium americanum* infected with *Ustilago heufleri* treated

with an antiserum directed against the fimbriae of *U. violacea* Fuckel and other fungi were then treated with protein-A-gold complexes to detect the presence and location of fimbrial antigens following transmission or scanning electron microscopy. The infected leaf sections were heavily labelled with gold particles indicating the presence of fimbrial antigens, whereas the control preparations showed only a low background level of labelling. Gold particles were detected on the sections of hyphae, on haustoria and on the nearby plant cells. The intensity of labelling was much higher for *P. hyoscyami* f.sp. *tabacina* than for *U. heufleri* and was particularly high in the walls of the former species. Relatively high levels of labelling occurred over the cells of infected host tissues, but little or low occurred over the cells of uninfected host tissues or of the infected host tissues treated with a range of serological controls. This level of labelling was not associated with specific host structures in *P. hyoscyami* , but was frequently associated with the chloroplast in *U. heufleri*. The antigens detected inside the host plant cells appeared to indicate that fungal fimbrial protein, either as polymerised fibrils or as isolated sub-units, could penetrate the host plasma membrane and therefore entered the host cytoplasm.(Day *et al.*, 1986).

Hyphae of *Verticillium dahliae* were detected in cotton root tissue with an ELISA. A soluble protein extract of *V.dahliae* was used to prepare a specific rabbit antiserum. The reaction of this rabbit antibody to the hyphae of *V. dahliae* was detected with an alkaline phosphatase antirabbit IgG conjugate that hydrolyzed the substrate, napthol-AS-phosphate, to a product that reacted with a diazonium salt, yielding a colored precipitate outlining the fungal hyphae. The hyphae were readily observed on and in the root cortex of the host using a dissecting microscope (Gerik *et al*, 1987).

Gerik and Huisman (1988) further observed that colony density of *V.dahliae* increased with distance from the root tip, with the maximal density occurring more than 1 cm from the root apex. Colony densities at distances more than 1 cm 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 toward the stele. Hyphae of *V.dahliae* also were found within numerous cortical cells. Colonies of *Fusarium oxysporum* similarly stained, were found to be mostly confined to the root surface and the outer cortex.

In enzyme-linked immunosorbent assay, antiserum raised against pooled mycelial suspensions from five isolates (designated pf 1, pf 2, pf 3, pf 10 and pf 11) of *Phytophthora fragariae* detected homologous soluble antigens at protein concentrations as low as 2 ng/ml (Mohan ; 1988) Fungal antigens could also be detected in extracts of strawberry plants infected with *P. fragariae.* Root extracts prepared from the alpine strawberry *Fragaria vesca* and *F. ananassa* cv. Cambridge Favourite infected with any of the five isolates studied produced strong reactions in ELISA. In *F. vesca*, ELISA positive material was detectable 6-8 days after inoculation before macroscopic symptoms appeared. The cultivar Red Gauntlet, resistant to 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 the corresponding extracts of plants inoculated with avirulent isolates.

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

Amouzou-Alladaye *et al.* (1988) reported that the antiserum obtained by injecting rabbits with mycelial protein extracts of one strain of *Phytophthora fragariae* had a dilution end point of 1:64 in double diffusion and 1/512,000 in indirect ELISA. This serum could detect 11 different strains of *P. fragariae* in pure culture and the pathogen in naturally infected or inoculated roots. Although the sensitivities of direct DAS and indirect ELISA were comparable, the direct DAS-ELISA was more specific for the detection of *P.fragariae* in strawberry roots. The antiserum failed to react with 18 fungal species isolated from underground parts of strawberry but reacted with some strains of *P. cactorum* , which parasitized only rhizomes but not roots, and *Pythium middletonii*, which was isolated sometimes in association with *P. fragariae* from strawberry roots. In inoculated strawberry

roots *P. fragariae* was detected reliably by ELISA several days before oospores were found and before symptoms developed. Thus, direct DAS-ELISA may be useful for early detection of infection and for the detection of latent infections of strawberry plants by *P. fragariae.*

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

An enzyme immunosorbent assay (ELISA) for isomarticin, a napthazarin toxin produced by *Fusarium solani* was developed by Phelps *et al*, (1989). A carbodimide procedure was used to couple the hapten isomarticin to BSA for the immunogen and to alkaline phosphatase for enzyme linked tracer. The resulting assay had a detection limit of 2 ng/ml for isomerticin, other napthazarin toxins were detectable at less than 10 ng per well in ELISA plates. The assay was specific for napthazarine. The cross reactivity with a number of phenolic compounds including the closely related napthoquinones was 3 order of magnitude less sensitive.

Gleason *et al* (1989) used freeze dried powdered mycelium of *Phomopsis longicola*, seed decay pathogen, in an indirect ELISA and an immunoblot assay for detecting seedborne infection. Antigen of *P. longicola* was detected by indirect ELISA in as little as 250 ng of dried mycelium/ml of extract. The antiserum reacted with mycelium preparation of *Diaporthe*

phaseolarum var. *sojae* showed comparatively little or no reaction when tested against 7 other seed borne fungi. *P.longicola* could be detected in individual symptomless infected seeds. A single infected seed coat could not be readily detected by indirect ELISA. Results of immunoblot assay for incidence of *P.longicola* and *D. phaseolarum* var. *sojae* in halved seeds from 10 seed lots correlated ($P< 0.001$) with agar plate bioassays 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 with agar plate bioassay but strongly ($P< 0.01$) with incidence of symptomatic seeds. Because immunoblot assays detected only viable *P. longicola* and ELISA did not discriminate between live and dead fungus, the authors concluded that former would be a better indicator of pathogen activity on seeds after planting. Werres and Casper (1987) detected *Phytophthora fragariae* in roots of strawberry cultivar 'Teniva' by ELISA, even before symptoms were detected microscopically.

Serological detection of *Plasmodiophora brassicae* by dot immunobinding and visualization of the serological reactions by scanning electron microscopy was also done (Large, 1989). A semipurified suspension of spores of *P. brassicae* was used as antigens, obtained by filtration and Percoll gradient centrifugation of infected roots. Crude root suspension samples were bound to a nitrocellulose paper and tested by dot immunobinding assay. 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. Noraml serum did not give rise to a coating on the resting spores. The antiserum against *P.brassicae* did not react with surface antigens of resting spores of *Polymyxa.* Further no cross reaction with other root pathogens was observed.

A double antibody ELISA to detect *Pythium* blight in turfgrass, with a monoclonal antibody directed against *Pythium* sp. has been developed. The monoclonal antibody was the product of hybridoma cell line PAS 111F11, produced using *P.aphanidermatum* as immunogen. The antibody bound *P.aphanidermatum, P.graminicola, P. myristylum* and *P. ultimum* all of which were involved with *Pythium* blight. *Pythium* spp. were detected in inoculated and naturally infected turfgrass samples, and the color intensity of the final reaction product of the immunoassay was directly related to the level of disease present. No cross reactivity was observed with other common turf grass pathogens (Miller *et al.,* 1989). Antiserum (anti-PfM) raised against mycelial suspensions of *Phytophthora fragariae* isolates

reacted strongly with antigen 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 (Mohan, 1989). He however, reported that anti-PfM could not be made specific for *P.fragariae* because it was raised against components shown to be antigenically similar in all *Phytophthora* species tested. However, immunoblotting with the affinity purified anti-PfM produced distinct patterns for *P.fragariae, P.erythroseptica* and *P.cactorum* : three serotypes were identified for the latter species. It was concluded that this antiserum might prove useful in classifying *Phytophthora* species.

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

A polyclonal antiserum, prepared in a rabbit immunized with a mycelium extract of *Phytophthora infestans* reacted in an enzyme-linked immunosorbent assay (ELISA) with mycelial extracts of two *Phytophthora* species but not with those of 10 other 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).

Ricker et al, (1991) detected water soluble antigens produced by *Botrytis cinerea* in picked and naturally infected grape juice by using an enzyme immunoassay with an indirect format of antibody HRPO conjugated bound to polyclonal rabbit antibodies directed against *B.cinerea* (anti Bc IgG). Protein A purified gamma globulin from an early bled antiserum (803.7), which reacted primarily with low molecular weight compounds present only in extracts of *B.cinerea*, was used to specifically detect *B.cinerea* and quantify levels of infection in juice from infected grape berries. Late bled higher titre antiserum (803-19), which cross reacted with proteins and carbohydrates present in extracts from species of *Botrytis, Aspergillus, Penicillium* and *Uncinula*, was used to quantify the levels of rot caused

by presence of multiple fungi. Minimum detectable levels of infections, based on mixture of clean and infected juice, were .25-.5% with (803-7)/IgG, and 0.2% with 803-19 IgG. Cross reactivity of anti Bc. IgG with extracted antigens (water soluble) from sterile and reproductive strains of several fungi was negligible in early bled antiserum and increased in subsequent collections. The increase in cross reactivity in late bled antiserum corresponded with an increase in the overall serum titres for anti Bc IgG to antigens from *B.cinerea.*

Polyclonal antisera prepared against purified mycelium proteins from *Verticillium dahliae* , the predominant fungus species in the potato early drying complex was tested against crude mycelial preparations of *Verticillium* spp. using indirect ELISA (Sundaram, 1991). It reacted positively with 11 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 *Colletotrichum* sp. from potato or with on isolate of *Rhizoctonia solani* from sugarbeet. Double antibody sandwich ELISA, using polyclonal antisera, detected *V.dahliae* and *V.allo-atrum* in infected roots and stems of potato.

Two commercial serological assay kits were compared to a culture plate method for detection of *Phytophthora cinnamomi* in root samples from infected azaleas by Benson (1991). Both the multiwell E kit and the rapid assay F kit detected *P. cinnamoni* on azalea roots beginning 1 week after inoculation. Agreement between immunoassay kits and culture plate results for detection of *P.cinnamomi* was not consistent beginning 3-5 week after inoculation. Root symptoms, but not foliar symptoms, of *Phytophthora* root rot were evident during this period. There was a positive correlation between root rot severity in green-house trials and root sample absorbance (multiwell) or meter reading (rapid assay) but not between symptom severity and immunoassay results. The multiwell kit detected *P.cinnamomi* in root samples containing as little as 1.0% infected root tissue. In a commercial nursery survey, 5 and 15% of the azalea root samples of two nurseries had positive ELISA values that were unconfirmed by culture plate. The rapid assay kit detected *P.cinnamomi* , was easy to use, and gave results in a short time.

Methods for sampling turf grass tissue were compared for their effectiveness in monitoring *Pythium* blight epidermis with enzyme-linked immunosorbent assay (ELISA). Samples consisted of whole plants picked by hand and assayed as whole plants; whole plants sectioned into lower, middle and upper strata components; leaf clippings collected with a reel mower set at a 1.2 cm cutting height. ELISA readings for mowed samples generally

matched those for whole plucked samples. Fluctuations in detectable *Pythium* antigens were most pronounced on the uppermost stratum compared with moderate to very little change in ELISA readings for the two lower strata. Several episodes of *Pythium* antigen increase were detected by ELISA assays of mowed samples, although signs and symptoms of *Pythium* blight were not evident. However, increase in ELISA readings for *Pythium* blight were not evident . However, increase in ELISA readings for *Pythium* coincided with, but did not generally precede, the onset of blight symptoms with a 2 to 3 day sampling interval, Shane (1991) concluded that antibody aided detection was useful for verification of diagnosis and determination of general *Pythium* population fluctuations, but was not satisfactory for advanced detection of blight epidemics.

Bossi and Dewey (1992) developed a monoclonal antibody - based immunodetection assay for *Botrytis cinerea* using three hybridoma cell lines secreting antibodies that specifically recognize *Botrytis cinerea* and *B. fabae* but not *B. allii,* in strawberries. Supernatants from the three specific cell lines recognized mycelial fragments, saline extracts of mycelia and germinating conidia by both ELISA and immunofluorescence. Recognition of non-germinating spores was poor. Supernatants from the specific cell lines did not recognize other fungi normally involved in post-harvest spoilage of fruits and vegetables. Supernatants from KH4 gave the lowest background values with healthy tissue. Indirect evidence from heat, protease and periodate treatment of the antigens indicated that antibodies from all three specific cell lines recognized carbohydrate epitopes on a glycoprotein.

Results of conventional isolation techniques for *Pythium violae* were compared with the assay of cavity spot lesions using polyclonal antibodies raised to *P. violae* or *P. sulcatum*, in competition ELISA (Lyons and White, 1992). Where lesions were artificially induced the test confirmed which pathogen was causal. With cavities developed on the field grown carrots *P. violae* again predominated and the ELISA confirmed this. In one sample *P. sulcatum* was also isolated from a small number of lesions and was not detected in ELISA. The competition ELISA did not indicate presence of either *Pythium* in a range of non-cavity spot lesions from which attempts at isolation were also negative.

A double antibody sandwich ELISA test was developed for the detection of *Pseudocercosporella herpotrichoides* using a highly specific monoclonal antibody pH-10 as the capture antibody and genus specific polyclonal rabbit antisera as test antibody. The assay recognized extracts from plants both artificially and naturally infected with *P. herpotrichoides*, at least three fold higher absorbance values with extracts *P. herpotrichoides*

infected tissue than with extracts from healthy tissues. The assay tested positiuively with all isolates of *P.herpotrichoides* including W type and R type isolates. The assay could be used to detect the pathogen in presymptomatic infected seedlings. The immunogen used was a mycelial extract from high molecular weight proteins and glycoproteins were removed by SAS. The high mol. wt. 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 dilution (Priestley and Dewey, 1993).

Linfield (1993) elicited polyclonal antiserum against a strain of *Fusarium oxysporum* f.sp. *narcissi* (GCRI 80/26) and a specific and sensitive enzyme-linked immunosorbent assay was developed. Antiserum raised to cell-wall fractions gave better recognition than that to cytoplasmic fractions. Recognition was equally good in artificially and naturally infected bulbs. Little cross reactivity in bulb tissue was shown by three other bulb-rotting fungi. Nine isolates of *F.oxysporum* f.sp. *narcissi* from a wide geographic area gave similar results in an indirect ELISA of mycelial extracts, although some 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 pathogen on selective medium.

New monoclonal antibody (MAb) raised against haustorial complexes (UB10) isolated from pea leaves infected with the powdery mildew fungus *Erysiphe pisi* recognised a 45KDa N-linked glycoprotein which was specially located in the haustorial plasma membrane. This glycoprotein was clearly distinct from a previously characterised 62 KDa plasma membrane (identified with MAb UB8) which was also specially located in the haustorial plasma membrane. These antibodies were used, along with MAb UB7 which binds to a major 62 KDa glycoprotein in the cell wall and plasma membrane of both haustoria and surface hyphae, to label haustoria within epidermal strips from infected pea leaves using indirect immunofluorescence. Results showed that all three glycoproteins recognized by MAbs expressed early in haustorial development (Mackie *et al.* 1993). Molecular differentiation in the extra haustorial membrane of pea powdery mildew haustoria of early and late stage development was subsequently focussed.

Lherminer *et al* (1994) used an indirect ELISA to monitor the distribution of a mycoplasma like organism (MLO), causal agent of grapevine yellow disease in the experimental host plant *Vicia faba*, during the course of infection after inoculation by leafhopper *Euscelidius variegatus*. Post-embedding collodial gold indirect labelling was developed to identify, the various forms of MLO in the different parts of the plant by TEM. Silver enhancement of the gold probe gave accurate histological and cellular location of MLOs in tissue sections by light microscopy. Both ELISA and immunolocalization first detected MLO in roots 17 days after inoculation. ELISA indicated the occurrence of a multiplication phase in the roots 17-24 days after inoculation. MLO then reached the collar and systemically colonized the basal axillary shoot. They preferentially multiplied in this apical area. MLO bodies were located in mature seive tubes and in non-functional phloem cells on the periphery of the vascular bundles, and necrotic cells were observed in the phloem tissue. Electron dense and distorted MLOs were identified in these collapsed cells.

Polyclonal mouse ascites antibodies were raised against soluble protein extracts of chlamydospores and mycelium. The IgG fraction was purified and biotin labelled to device a fungal capture sandwich ELISA. ELISA detected both brown and grey cultural types of *T.basicola* and had negligible cross reactivity with other soil borne fungi. The minimum detection limit of ELISA was between 1 and 20 ng of *T.basicola* protein depending on the assay. *T.basicola* could be detected in cotton roots two days after inoculation. At this time, initial symptoms were apparent. The antibody also was used to observe *T.basicola* on cotton roots with immunofluorescence microscopy (Holtz *et. al.*, 1994).

Polyclonal antibodies produced against culture filtrate and mycelial extracts immunogen preparations from the soybean (*Glycine* max) fungal pathogen *Phomopsis longicolla* were purified to the immunoglobulin fraction and tested in indirect ELISA and in direct DAS ELISA (Brill *et.al.*, 1994). The PABs raised to culture filtrate were more specific but less active in binding to members of the *Diaparthe-Phomopsis* complex than were those 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 rabit with culture filtrate over an extended time resulted in maximum anti *P.longicolla* activity after three immunization, and the activity became constant against most members of the complex at the same time. Reactivity to some culture of *P.longicolla* was undetectable following the fourth and fifth immunizations, whereas reactivity to all of other cultures of the complex remained high.

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

An immunoassay for the detection of phomopsin was used by Williamson *et al,* (1995) to detect levels of the mycotoxin in epidermal peels from resistant and susceptible lupin cultivars asymptomatically infected with *Diaporthe toxica.* Quantifiable levels (>6,25μg/kg) of phomopsin were detected in susceptible lupin cultivars, but not in very resistant breeding lines or in the control. These differences reflects the differences in resistance observed in microscopical assays and mature plants in the field.

Wakeham and White (1996) raised polyclonal antisera against whole (coded : 16/2), and sonicated (coded : 15/2) resting spores of *Plasmodiophora brassicae* as well as soluble components prepared by 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 1×10^7 resting spores g^{-1} in soil. With Western blotting the lower limit of detection with antiserum 15/2 was 1×10^5. This antiserum showed the greatest sensitivity in a dip-stick assay, indirect ELISA and indirect immunofluorescence, for all of which there was a limit of detection of 1×10^2. The indirect ELISA was successful only after the substitution of alkaline phosphatase by protein. A conjugated horseradish peroxidase. Of the assays tested, indirect immunofluorescence appears to be the most rapid and amenable assay for the detection in soil of low levels of resting spores of *P.brassicae.*

Polyclonal antiserum was raised against mycelial extract of *Pestalotiopsis theae* and immunoglobulin fractions were purified by ammonium sulfate fractionation and

chromatography on DEAE sephadex. In ELISA, antiserum dilution upto 1:16000 detected homologous antigen at 5 mg/L concentration and at 1:125 antiserum dilution fungal antigens could be detected at concentration as low as 25 μg/L. In 15 varieties of tea tested, from Darjeeling, UPASI and Tocklai Research Stations, absorbance values of infected leaf extracts were significantly higher than those of healthy extracts at 40 mg/L concentration in indirect ELISA. ELISA positive material was detected in tea leaves as early as 12 h after inoculation with *P.theae.* At serum dilution upto 1:125, pathogen could be detected in infected leaf extracts upto 2 mg/L concentration. The results demonstrated that ELISA can be used for early detection of *P.theae* in leaf tissues even at very low level of infection (Chakraborty et al, 1996).

Serological differentiation among fungal species

It is possible to differentiate fungal species on the basis of serological relationship. Serological cross reactivity within species is found to occur in several cases and this is an area of research which is also receiving much attention.

Three species of the genus *Phytophthora* were studied serologically by means of 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 hyphae. Species specific sera were obtained and proved useful and efficient for the identification of *P.cactorum, P.cinnamoni* and *P.erythroseptica* (Burrell *et al*, 1966).

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

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

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

The serological cross-reactivity of *Sporothrix schenckii* with various unrelated fungi was investigated. A strain specific antiserum prepared against the mature hyphae of *Fusarium culmorum* was used by Hornok and Jagicza (1973) for direct and indirect methods of the fluorescent antibody staining technique. There was a significant difference in the intensity of fluorescence between the mycelia of the homologous *F.culmorum* strain and those of the heterologous *F.graminearum* and *F.culmorum* strains. All the other *Fusarium* species tested showed no detectable fluorescence. The heterologous strains of *F.culmorum* and *F.graminearum* could not be distinguished. No significant difference was found between the 'culmorum' strains from different host plants. The serum specific for *F.culmorum* and *F.graminearum* was suitable to separate these species from other fungi.

Soluble proteins from the mycelia of 30 isolates of *Phytophthora cinnamoni* collected by Gill and Zent (1977) from 17 different hosts and from widely separated geographic locations 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 making it possible to

identify them. With one exception, there was little or no variation in the protein patterns within the isolates of *P.cinnamoni*. Also, identical or nearly identical protein patterns of each species were obtained regardless of date of isolation, host on geographic locality. No differences in the sprotein patterns were seen between the mating types of *P.cinnamoni*.

Savage and Sall (1981) used radioimmunosorbent assay (RISA) for somatic antigens of the pathogen *Botrytis cinerea* to detect the presence of *Botrytis* antigens in homogenized samples. As little as 100 ng of original fungal dry weight could be detected and the sensitivity curve was log-log linear in response upto 10 mg. The assay was highly specific for *B.cinerea* although some reaction was obtained with other members of Sclerotiniaceae. *B.allii* showed 48% reactivity relative to *B.cinerea,* and species of *Sclerotinia* showed 10-24% reactivity. All other fungi tested showed less than 0.1% reactivity. The usefulness of the assay for detection of the fungus within host tissue was demonstrated by the high correlation of the assay results with an estimation of rot weight from field-infected lots.

Ishizaki *et al* (1981) by use of immunodiffusion test. A rabbit anti-*S.schenckii* sera was obtained which reacted with *Cladosporium werneckii, C.carrionii, C.bantianum, Coccidioides immitis, Phialophora geanselmei, P. gougerotii, P.dermatitidis, Fonsecaea pedrosoi, Aspergillus fumigatus, Histoplasma capsulatum* and *Trichophyton mentegrophytes,* but not with *Saccharomyces cerevisiae* antigens. The serological determination responsible for the cross reactions were suggested to be galactosyl residues.

Four formae speciales of *Fusarium oxysporum (dianthi, melonis, pisi, lycopersici*) and three physiological races (1,2,3) of *F.oxysporum* f.sp. *melonis* have also been differentiated using specific antisera and the techniques of double diffusion and radial double diffusion in agar (Iannelli *et. al.* 1982). 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.

Immunologic analyses of teliospore surfaces by Banowetz *et al* (1984) using polyclonal antisera and monoclonal antibodies indicated that *Tilletia controversa* and *Tilletia caries* were very similar. Although two polysaccharide antigens were present in teliospore extracts, these components appeared to be immunologically identical in both species and no protein antigens were demonstrated by either electrophoretic or immunologic means. Spectrophotometric analyses of surface extracts also indicated no extractable protein. None of seven fluorescein-labelled lectins bound these teliospores, even after the spores were treated

with 8M Urea to enhance exposure of potential lectin-binding sites. An antibody 'double sandwich' enzyme immuno-assay demonstrated quantitative differences in the numbers of certain monoclonal antibody binding sites of the two fungi although these differences did not provide a basis of the unambiguous detection of either bunt species in contaminated wheat shipments. No quantitative distinction of the two species was found by any of the methods used in this study.

Immunological comparison between isolates of *Botrytis cinerea* by crossed immunoelectrophoresis has been done by Ala-El-Dein and El-Kady (1985). CIE techniques showed that the tested isolates were serologically different; some antigens were specific for each isolate, *Botrytis cinerea* isolate no.1 had four specific antigens; these antigens were absent in the other isolates. At least sixteen antigens were common in the isolates tested. Some isolates were serologically similar when tested by double gel diffusion test, while they were distinguishable when CIE techniques were used. Numbers of precitptin peaks obtained with CIE techaniques were more than double the number of precipitin lines detected with double gel diffusion test. Results revealed that CIE techniques could be used as valuable analytical tools in resolving the spectrum of antigens present in *Botrytis cinerea* isolates. They also compared the antigenic structures of *Botrytis cinerea, B.tulipae, B.paeoniae* and *B.allii* isolates by using CIE techniques. Antisera against antigens of these isolates gave 24, 15, 20 and 15 precipitin peaks, respectively, when analyzed in homologous reactions, CIE with an intermediate gel and CIE with antibody absorption *in situ* reacted that each isolate was serologically different from the other and had species-specific antigens. Eight antigens distinguished *Botrytis cinerea* from the other species of *Botrytis*, these were present only in the former species. *Botrytis allii* had less common antigens than the other species. These results showed that CIE techniques were more successful and sensitive tools for these comparative studies than the double gel diffusion test.

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

Cross-reactivity between antisera produced against fimbriae of either *Ustilago violacea* (AU) or of *Rhodotorula rubra (*AR) and cell surface proteins of two ascomycete fungi,

Ascocalyx abietina and *Ophiostoma ulmi* was revealed by means of dot-immunobinding and immunocytochemical methods. Following treatment with antiserum AR, the walls, septa, and plasma membrane of *A.abietina* and *O.ulmi* cells were appreciably 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 AU while all other structures did not. No significant labelling with this antiserum occurred over cells of *O.ulmi* indicating that they either lacked these antigens or that these were more easily removed during the fixation process (Benhamou *et al,* 1986).

The agglutinating effect of 5 lectins having different sugar binding specificities on the conidia of a variety of strains from 8 *Fusarium* sp. were examined by Christenzio *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-acetyl D-galactose amine inhibited the agglutination induced by concanavalin A and *Helix pomatia* agglutinin, respectively. Conidia of the other species of *Fusarium* examined (*F.culmorum, F.sambucinum, F.graminearum, F.avenaceium, F.moniliforme* and *F.xylarioides*) were not agglutinated by either lectin. No conidia from the *Fusarium* sp. were agglutinated by wheat germ agglutinin, potato lectin, *Ulex uropeas* agglutinin-1. These results have provided some insight into the chemical nature of the outermost layer of the fusaria conidia and this information may be of taxonomic significance.

The potential of polyclonal antisera and monoclonal antibodies to differentiate the EAN and NAN aggressive subgroups of *Ophiostoma ulmi* was explored by Dewey *et al,* (1989). Polyclonal antisera, when tested by ELISA, cross reacted widely with unrelated species and failed to distinguish between the two aggressive subgroups but small quantitative differences were found, particularly between antigens secreted overnight by EAN and NAN germlings. Out of 33 cell lines that secreted monoclonal antibodies specific to *O.ulmi*, one third were non-specific, 11 were specific either to species or subspecies. Two cell lines differentiated mycelial antigens of the aggressive isolates of *O.ulmi* from those of the non-aggressive subgroup, but not antigens from surface washings. Only quantitative differences were detected between the EAN and NAN aggressive subgroups. Almost all the monoclonal antibodies and antiserum recognized antigens present in surface washings of cultures on solid medium, in cell free extracts of mycelial homogenates, in cell free culture fluids, and in substances secreted overnight by germinating spores. Most of the monoclonal antibodies appeared to have potential diagnostic value, since they gave readings two-fold to ten-fold higher with extracts from diseased than from healthy tissue.

Soluble proteins from 34 isolates representing 16 species of the genus *Phytophthora* from different geographic locations and host plants, when analysed by two dimensional electrophoresis, were resolved into 100 to 200 protein spots with different densities (Masago *et al*, 1989). 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 highly similar, if not identical, among isolates of the same species regardless of their geographic origin, original host plant, mating type, or virulence race. Authors have suggested that the two dimensional electrophoretic patterns, being species-specific may be a useful measure to differentiate and identify species of the genus *Phytophthora.*

Fuhrmann *et al.* (1989) tested antisera produced against *Penicillium verrucosum* var. *verrucosum* for their reactivity with 44 strains of moulds by immunofluorescence and by indirect enzyme linked immunosorbent assay. Antigenically *P. verrucosum* var. *verrucosum* (subgenus *Penicillium*) appeared 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.*

Kitagawa *et al* (1989) have developed competition types of 2 novel enzyme linked immunosorbent assays (ELISA) for specific detection of *Fusarium oxysporum* f.sp. *cucumerinum* as well as for general detection of 10 strains of common *Fusarium* sp. that show specific pathogenecities to different plants. Antiserum against a strain of *F.oxysporum* f.sp. *cucumerinum* (F 504) 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 labelled anti rabbit IgG as the secondary antibody and cell fragments of the strain to amino dylark balls as the solid phase antigens. This assay was specific for strain F 504 and showed little cross reactivity with 9 other strains of *Fusarium* sp. including strains 501 of *F.oxysporum* f.sp. *cucumerinum.* Strain F 501 possessed pathogenicity against cucumber similar to that of strain F 504 although slight differences have been observed between these two strains regarding their spore formation and pigment production.

Antibodies of three isolates each of *Armillaria mellea, A.astoyae, A. tabescens and Lentinula edodas* were tested against the different isolates by an enzyme-linked immunosorbent assay (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 the homologous species from isolates of the heterologous species. The specificity of the antibodies present in eggs was dependent on time elapsed since immunization. Eggs laid 3 weeks after immunization with *Armillaria* species 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. (Burdsall, 1990).

Monoclonal antibody (Mab) LK50 was developed against *Leptosphaeria korrae* strain ATCC 56289 (Nameth et al, 1990). The antibody was capable of detecting *L.korrae* in cultures and in the naturally infected bluegrass samples from three states. In cross reactivity tests using indirect ELISA, MAb reacted positively to all 24 isolates of *L.korrae.* MAb reacted negatively with 38 of 42 isolates of related and non related fungi and negatively to apparently healthy grass. The limit of detection was less than 2 μg/ml of lyophilyzed mycelia

Daniel and Nilsson (1991) reported that polyclonal antiserum raised against mycelial extracts of the rot fungus *Phialophora mutabilis* reacted strongly with its homologous antigen and cross reacted strongly to moderately with six other *Phialophora* soft. rot spp. in ELISA. With the exception of *Ceretocystis* sp. the serum reacted weakly or not at all with 11 other mold, blue and rot fungi. The antiserum cross reacted strongly with antigens in extracellular filtrates from *P.mutabilis* cultures that contained about 40 ng/ml protein.. Ultrastructural and immunocytochemical studies on wood degraded by *P.mutabilis* showed specific localization of the antibody on the fungal cell wall and certain intracellular structures. The antiserum was assessed by ELISA for detecting the presence of the fungus and soft rot in untreated and preservative treated wood blocks of pine and birch degraded for periods of 1-12 months. *P.mutabilis* was detected in samples from all wood blocks degraded to low or high wt. loss. Highest ELISA readings were recorded for wood blocks with highest substrate losses and vice versa.

Extracts from *Fusarium oxysporum* (F.O) and *F.oxysporum* var. *redolens* (F.O.r.) isolates were compared by means of electrophoresis and cross immunoelectrophoresis (Rotej Guranowska and Walko, 1991). Both fungi appeared almost identical serologically. Relative amounts of their corresponding proteins differed but the quantitative patterns of the proteins were nearly the same. With the anti-*F.oxysporum* var. *redolens* serum, only one specific antigen was detected in the extracts from *F.oxysporum* var. *redolens* isolate. Although the

results obtained indicated a strong similarity between *F.oxysporum* and *F.oxysporum* var. *redolens.* The authors considered that they could not be separated into 2 species.

The sensitivity of a *Phytophthora* specific immunoassay kit was tested by Pscheidt (1992) 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 absorbances relative to other species were obtained from *P.cinnamomi* and *P.megasperma* (originally isolated from cherry). 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 culture of *Phytophthora* sp. isolated from these samples. Cross-reactions occurred with several *Pythium* spp. isolated from clinic samples and with several specimens infected with *Peronospora* spp. Other samples without typical *Phytophthora* symptoms but associated with other pathogens did not produce a positive reaction with the immunoassay.

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