Discussion

A complex and variable developmental pattern between host and parasite is initiated by the invasion of the host tissue by pathogen which continues throughout their course of coexistance. Differences in physiological responses and morphological structures of various host genotypes affect their susceptibility or resistance to invasion and its consequences while similar variation in pathogens influence their growth rate and virulence (Loomis and Adams, 1983). In nature,a multitude of potential invadors are present in the plant environment. In spite of this , disease is still the exception rather than the rule. This is because a potential pathogen in order to establish a compatible interaction must recognize features of the plant which signals the suitability of that plant for parasitism. At the same time the potential host may be able to detect or recognize a potential fungal pathogen or nonself and use the initial act of recognition to trigger a range of induced resistance mechanisms (Callow, 1982, 1983; Purkayastha, 1994). Plants thus have evolved immune mechanisms of various types by which they can counter the advance of foreign organisms. The result is that, disease tends to be specific, a given pathogen usually infecting a distinct range of host plant.

In the host pathogen interaction therefore, the initial cellular recognition is followed by communication between its components, whereby information is transmitted to and received by cell to signals or instructions the cell can understand. This exchange of information are generally mediated by soluble antigens located on or near the cell surface (Chakraborty, 1988).

At the onset of the present study varietal resistance tests of 37 varieties of tea released by Tocklai Experimental Station, Jorhat, Assam; Darjeeling Tea Research Centre, Kurseong, Darjeeling; UPASI Tea Research Centre, Valparai, Tamilnadu, against the brown blight pathogen, *G. cingulata* was carried out by detached leaf and cut shoot inoculation techniques. Responses exhibited by the different varieties were essentially the same in both the inoculation techniques. Of the 37 varieties tested, UPASI 9 and BT-15 were the most and least susceptible respectively. Yanase and Takeda (1987) detected resistance of tea plants to grey blight disease caused by *Pestalotia longiseta* by cut shoot method.

Fungal plant pathogens invade host plant cells with a variety of specialised infection structures of which, for most fungi the appressorium is developmentally the first and most important infection structure formed in preparation of host colonization (Hoch and Staples; 1987). It must be positioned at an appropriate site on the host in the most advantageous way for subsequent infection to occur. In many rust fungi positioning of the appressorium is most critical because invasion of the host can occur only through the stomata (Staples and Macko, 1984). It has been reported by Ando and Hamaya (1987) that infection of tea by the anthracnose fungus *Gloeosporium theae-sinensis* could only occur through the trichomes of young leaves. In many cases this pathogen was inhibited from gaining entrance by a callosity which was produced by swelling of the trichome cell wall inward in a way that enveloped and preceded the invading hypha. Formation of appressoria being the first step in establishing the disease, the factors affecting this process are of vital importance in deciding the fate of the pathogen in initial stages (Purkayastha and Menon, 1981). For *G. cingulata* which is able to infect only through wounds (Baxter, 1974; Bertus, 1974; Dickens and Cook, 1989), the positioning of an appressorium assumes great significance. Chakraborty *et. al* (1995a) studied the factors influencing spore germination, appressoria formation and disease development in *Camellia sinensis* by *Glomerella cingulata.* They reported that spore germination and appressoria formation were optimum at a temparature of 25^{0}C, pH 5.0, 7h light/day regime and a 24h incubation period. In the present investigation optimum condition necessary for growth of *G. cingulata* was tested *in vitro*. Maximum growth occurred at an incubation period of 10 days, pH 6.5 and using maltose and potassium nitrate as carbon and nitrogen sources respectively. Sporulution behaviour varied with the different media on which the fungus was grown.

During host parasite interaction even when coincidence of location and time is provided and supplemented with optimum conditions for the development of the pathogen, parasitic relationship can only be established if the host recognizes the pathogen on the one hand, and the pathogen can overcome the various defence mechanisms of the host, on the other hand

whenever an intimate and continuing association of cells of host and pathogen occur it has been observed that partners of this association have a unique serological resemblance to one another involving one or more antigenic determinants. The presence of cross reactive antigen (CRA) between plant host and their parasites and the concept that these antigens might be involved in determining the degree of compatibility in such interactions have been reviewed by several authors (Devay et. al, 1972; Devay and Adler, 1976; Kalyanasundaram, 1978; Chakraborty, 1988; Purkayastha, 1989; Purkayastha et. al., 1991; Purkayastha, 1994). In the present study, leaf antigens of 37 tea varieties, 3 non hosts (*G. max* , *C. arietinum* and *C. japonicum*), 9 isolates of *G. cingulata* and one non pathogen of tea (*F. oxysporum*) were cross reacted separately with anti - *G. cingulata* antiserum. The presence of cross reactive antigens among *G. cingulata* (all isolates) and 16 varieties of tea (TV-18, 20, 22, 23, 25, 27, Teenali 17/1/54, UPASI 3, 9, 26, T-78, B-157, TTV_1 K1/1, B668 and B777) was evident in immunodiffusion test. No common antigenic substance was found between *G. cingulata* and 8 other varieties (TV-26, UPASI-2, P312,BT-15, AV-2, RR17, T-135 and CP-1). However, weak precipitin reaction was observed with antigens of 13 other varieties (TV-9, 28, 29, 30, TS-449, UPASI-8,17 BSS1, 2, 3, P-1258, BS/7A76 and HV-39). Cross reaction of anti- *G. cingulata* antiserum with antigens of non pathogens and non hosts failed to develop any precipitin band. Reciprocal cross reaction was also carried out with antisera of TV-18 (susceptible variety) and CP-1 (resistant variety). Presence of common antigens between *G. cingulata* and suceptible tea varieties were confirmed in the reciprocal cross reaction with the antiserum of TV-18. None of the isolates of *G. cingulata* developed any precipitin reaction with anti CP-1 antiserum. Non host species and non pathogens also fail to develop any precipitin band with either of the two antisera.

Several earlier studies have also implicated the importance of common antigens in host pathogen compatibility. The occurrence of CRA and their involvements in various host parasite combinations have been demostrated. These are flax and *Melamspora lini* (Doubly et. al., 1960), cotton and *Verticillium alboatrum* (Charudattan and DeVay, 1972) cotton and *Fusarium oxysporum* f. sp. *vasinfectum* (Charudattan and DeVay, 1970; Kalyanasundaram *et. al.*, 1975), sweet potato and *Ceratocystis fimbriatae* (DeVay *et. al.*, 1976), potato and *Phytophthora infestans* (Palmerley and Callow, 1978; Alba and DeVay, 1985), soybean and *Macrophomina phaseolina* (Chakraborty and Purkayasta, 1983), soybean and *colletotrichum dematium* var. *truncata* (Purkayastha and Banerjee, 1986), soybean and *Myrothecium roridum* (Ghosh and Purkayastha, 1990, coffe and *Hemiliea vastatrix* (Alba *et. al.*, 1983), groundnut and *Macrophomina phaseolina* (Purkayastha and Pradhan, 1994); tea and *Bipolaris carbonum* (Chakraborty and Saha, 1994). Present result also confirm the presence of CRA between *P.*

theae and susceptible tea varieties reported by Chakraborty *et. al.* (1995).

Results obtained in immunodiffusion tests were further confirmed by immunoelectrophoretic analysis with antigen and antisera preparations from tea leaves (CP-1 and TV-18) and *G. cingulata.* Reaction of anti - *G. cingulata* antiserum with homologous antigen revealed the presence of 6 precipitin arcs of which 2 were common with the susceptible varieties, UPASI 9, TV-18 and B- 157. No precipitin arc was formed between antiserum of *G. cingulata* and antigens of TV-26, UPASI 2 and CP-1 (resistant varieties), *G. max, C. arietinum* and *C. japonicum* (non hosts) and *F. oxysporum* (non pathogen). Following rocket immunoelectrophoresis a single precipitin line was discerned for TV-18 when reacted in the agarose gel containing *G. cingulata* antiserum. The present results are in agreement with the findings of previous workers (Chakraborty and Purkayastha), 1983; Purkayastha and Ghosal, 1987; Ghosh and Purkayastha, 1990). In the above studies Purkayastha and coworkers have examined various host pathogen/non pathogen combinations including cultivars of soybean, rice, jute, pigeon pea, bean and groundnut to find out the serological relationship with some fungal pathogens as well as non pathogens following immunodiffusion and immunoelectrophoretic tests. More than 50% combination between host and pathogen while no CRA could be detected between resistant host and respective pathogens. Chakraborty and Saha (1994b) have also reported similar findings with tea and *Bipolaris carbonum.*

In host parasite interactions failure to detect CRA by conventional serological techniques have been attributed to a number of factors including antigen treatment, method of extraction of antigen, culture of microbes and age of plants and tissue (DeVay and Adler, 1976; Chakraborty,1988). Alba *et. al.*, (1983) showed that urediospores of *Hemilia vastatrix* shared common antigenic determinants with coffee plants which was in contrast to their previous conclusions made with the same host parasite system (Alba et. al., 1973). This disagreement was attributed to the low concentration of antigenic preparations used in earlier investigation.

Among all available serological techniques for detection of CRA enzyme linked immunosorbent assay is probably the most sensitive (Alba and DeVay, 1985; Chakraborty and Saha, 1994). In the present study antisera were raised against antigenic preparation of *G. cingulata* from mycelia and cell wall separately. The antisera thus obtained were initially purified and the IgG fraction was used in all further tests. This was necessary to minimize non specific binding which may interfere with the actual antigen antibody reaction. At the onset the sensitivity of the assay was optimised. Homologous soluble antigens at a

concentration as low as 25ng/ml could be detected in indirect ELISA by both antisera. Positive results were also obtained at antisera dilutions of upto 1:16,000. Absorbance values in ELISA decreased with increased dilution. *Pythophthora fragariae* antigen at a concentration as low as 2ng/ml was detected in indirect ELISA using antiserum raised against pooled mycelial suspension of 5 *P. fragariae* races (Mohan, 1988). He suggested that this sensitivity may be because of the indirect ELISA method used; the direct double antibody sandwhich form of ELISA (Voller *et. al.*, 1976) detected antigen at protein concentration greater than 50ng/ml. The indirect method used 'native' antibodies rather than antibody conjugates required in the direct assays and hence avoided masking or impairing of antigenic binding sites by the conjugation procedure. Chakraborty et. al. (1996) also reported that antiserum raised against *Pestalotiopisis theae* could detect homologous antigen at 25 ng/ml. Antiserum dilution of upto 1:16,000 was effective for detection.

In the present study indirect ELISA readily detected CRA in semipurified antigen preparations of *G.cingulata* at a concentration of 40 μg/ml with 1:250 antiserum dilution. Alba and DeVay (1985) also detected CRA in crude preparations and in purified preparations from mycelia of *Phytophthora infestans* (races 4 and 1.2.3.4.7) using antisera of potato cultivars King Edward and Pentland Dell at concentrations lower than 50 μg/ml protein in indirect ELISA. Among the 37 tea varieties tested with antiserum of *G.cingulata* raised against either mycelial or cell wall preparations high absorbance values were obtained in reaction with antigenic preparations from UPASI 9, B-157 and TV-18 while CP-1, UPASI-2 and BT-15 showed lowest reactivity. In order to determine the observed cross reactivity between *G.cingulata* and suceptible varieties was specific, antigen preparations from non host (*G.max, C.arietinum* and *C.japonicum*) and non pathogen (*F.oxysporum*) were also assayed with antisera of mycelia and cell wall of *G.cingulata* (isolate GC-1), resistant (CP-1) and susceptible (TV-18) tea varieties and non pathogen (*F.oxysporum*). None of the above reactions showed any reactivity in ELISA. Since indirect ELISA test gave the same results in all three repetitions it appears that this observed antigenic disparities have some significance in the basic compatibility of host and pathogen (Chakraborty, 1988). In another experimental set up of DAC ELISA when antigens prepared from 3 categories of leaves (uppermost young, middle and mature leaves) each of 11 Tocklai varieties of tea were cross reacted with anti-*G.cingulata* antisera raised against both mycelia and cell wall, all three categories of leaves gave positive response. Recognition of leaf antigen with cell wall antiserum of *G.cingulata* was always better than the antiserum of the pathogen raised against mycelial preparations. No correlation could be established between ELISA responses and age of leaves. However, irrespective of varieties or categories absorbance values were always higher for antiserum raised against cell wall than mycelia of the pathogen.

Detection of major CRA shared between *G.cingulata* and tea leaves was comparable in both the formats of ELISA tested viz. double antibody sandwich (DAS) and direct antigen coated(DAC). Both the methods showed sensitivities with leaf extracts but DAC-ELISA was more specific. It appears from the results of studies on cross reactive antigen of *G.cingulata* and tea that common antigenicity between tea leaf and *G.cingulata* may be associated with susceptibility to brown blight disease.

Visible outcome of a compatible host pathogen interaction may be obtained in many cases only after several days of infection, by which time the pathogen would be well and truly established in the host tissues. In plant disease studies, therefore, it is necessary to have techniques by which the pathogen can be detected at a very early stage. Recent trends in detection of plant pathogenic fungi include the development of more rapid diagnostic techniques with high specificity for the target organism. These techniques can be used to detect fungi present in low amount in or on plant tissue and therefore in many cases the pathogen can be detected at a much earlier stage of disease development than was previously possible (Hansen and Wick, 1993). Various formats of ELISA using polyclonal antiserum has found widespread application in plant pathology and are routinely used for detection and identification purposes (Clark and Adams, 1977 ; Clark, 1981; Lommel *et. al.* 1982, Sundaram *et. al.*, 1991; Lyons and White, 1992).

Differential responses of the different varieties of tea to *G.cingulata* has been established by both pathogenicity test and cross reactivity test between *G.cingulata* and tea varieties. Following this, the ability of the antisera raised against both mycelial and cell wall preparations of *G.cingulata* to detect the pathogen in infected tea leaves was tested in ELISA. ELISA could detect infection in all the varieties tested irrespective of their susceptibility or resistance in other tests. Pathogen detection in the host tissue by ELISA have also been reported by a number of previous workers. These include the detection of *Pythium* species, *Rhizoctonia solani* and *Sclerotinia homeocarpa* in turfgrass (Miller *et. al.*, 1986; *Humicola laniginusa* in rice (Dewey *et.al.*, 1989), *Leptosphaeria korrae* in turfgrass (Nameth *et. al.*, 1990), *Phytophthora* species in a variety of woody ornamentals (MacDonald *et.al.*, 1990; Benson, 1991) and *Septoria nodorum* and *S.tritici* in wheat (Mittermeier *et. al.*, 1990, Peterson *et.al.* 1990). Mohan (1988) reported the ability of anti *P.fragariae* antiserum to detect infection in strawberry cultivars. *P. fragariae* infections were also detected readily in field infected samples of strawberry cultivars. Amouzou-Alladaye *et.al.* (1988) also reported the use of specific IgG of *Phytophthora fragariae* in DAS ELISA which constituted a method of early detection of the fungus in roots of inoculated plants. For the 5 varieties studied *P.fragariae* could be detected between 15 and 25 days after inoculation. In the present study natural brown blight infections could also be detected easily in ELISA.

To determine the earliest time at which infections could be detected extracts from *G.cingulata* inoculated tea leaves were prepared at 6 h intervals and tested against anti *G.cingulata* antiserum in ELISA. Infections could be detected as early as 6 h after inoculation in the susceptible variety (B 157) while in the resistant variety it could be detected either from 18 h onwards (by cell wall antiserum) or 30 h onwards (by mycelial antiserum).Thus,

ELISA could easily detect infections in the leaf tissues much earlier than the appearance of the visible symptoms which generally appeared after 48 h of inoculation with *G.cingulata.* This is in confirmity with the results of several previous authors who have reported that ELISA could detect pathogens in tissues (Linfield, 1993; Jamoux and Spire, 1994). In experiments conducted over 20 days with *Fragaria vesca,* Mohan (1988) showed that ELISA positive material was detectable 6-8 days after inoculation with *P.fragariae* when the plants were apparently still healthy.

Antisera raised against either mycelial antigen preparation or cell wall antigen preparation of *G.cingulata* could detect infections in tea leaves. In general antisera raised against cell wall preparations gave slightly higher absorbance values. El-Nashaar (1986) made polyclonal antisera to soluble hyphal antigens and to cell wall antigens. They found that the number of fungi that cross reacted was reduced considerably when the cell wall fraction was used as an immunogen instead of the soluble cytosol fraction. Gerik *et.al.* (1987) on the other hand stated that the antigen preparation containing fungal cell wall or cell wall materials could evoke antibodies to chitin or other cell wall polysaccharides common to most fungi. They obtained a more specific sera using only soluble fungal proteins as the antigen. Linfield (1993) again reported that antiserum raised to cell wall fraction of *F.oxysporum* f.sp. *narcissi,* gave better recognition than that to cytoplasmic fraction. Holtz *et.al.* (1994) produced polyclonal antisera to both the cell wall fraction and the soluble cytosol fraction of *Thielaviopsis basicola.* Both indirect ELISA and fungal capture sandwich ELISA revealed no difference in specificity of the cell wall and the cytosol antisera and the antibodies to each immunizing fraction reacted with the other fraction.

G.cingulata could be detected in ELISA upto a concentration of 2 μg/ml proteins in infected leaf extracts. Detection of infection in such low levels has also been previously reported by Mohan (1988) who showed that *P.fragariae* infected strawberry roots diluted 200-400 fold yielded positive reactions in ELISA. He suggested that infection levels 200-400 times less than those tested might be detectable. In the present study it is noteworthy that some amount of cross reactivity was always obtained between anti *G.cingulata* antiserum and healthy tea leaf extracts. This may be due to the presence, to some extent, of cross reactive antigens between the host and the pathogen.

Since the antiserum raised against *G.cingulata* could detect *G.cingulata* infections in tea leaves it was further decided to evaluate its specificity with regard to detection of the pathogen both *in vivo* and *in vitro*. Anti *G.cingulata* antiserum was shown to react positively with extracts of tea leaves artificially inoculated with *G.cingulata* but reactions with extracts of leaves inoculated with the pathogens were comparatively lower when tested in DAC ELISA.

Brown blight and grey blight symptoms are sometimes difficult to differentiate in the field since they are known to occur in close association in nature. The use of competition or inhibition ELISA for the recognition of specific microorganisms in mixed population from plant tissues has been reported (Alvarez, 1990). A competitive type of ELISA was therefore used in the present study to determine the predominant pathogen from mixed natural infections

Antiserum raised against *G.cingulata* gave positive results against antigen extracts of naturally infected tea leaves while antiserum raised against *P.theae* gave negative results in this test. This confirmed that the infection was mainly of brown blight with *G.cingulata* being the predominant pathogen. Lyons and White (1992) also used a competition ELISA to determine the predominant pathogen in cavity spot of carrots. They reported that *Pythium violae* was predominantly isolated from infected carrot tissues and competitive ELISA also confirmed *P.violae* as the causal agent and not *P. sulcatum.*

Cross reactivity of the antiserum raised against *G.cingulata* (isolate GC-1) was tested against 8 other isolates (GC-2 to 9). Since *G.cingulata* is a telomorph of *Colletotrichum gloeosporoides* the cross reactivity of the antiserum raised against *G.cingulata* with other species of *Colletotrichum* was tested. Cross reactivity was also tested with other foliar pathogen of tea. Results revealed that anti *G.cingulata* antiserum cross reacted to a greater or lesser degree with the different isolates but not with those of other species of *Colletotrichum* or other genera. Mohan (1989) showed that antiserum raised against mycelial suspension of *Phytophthora fragariae* (PfM) reacted strongly with antigen from several *Phytophthora* species. He observed that anti PfM could not be made specific for *P.fragariae* because it was raised to components shown to be antigenically similar in all *Phytophthora* species tested. Similar results with *P.fragariae* were also reported by Amouzou-Alladaye *et.al.* (1988). In their studies antibodies obtained with a strain of *P.fragariae* detected 11 different strains of pathogen in pure culture by both DAS and DAC ELISA. Harrison *et.al.* (1990) further reported that anti *P.infestans* γ-globulin reacted strongly with an extract of *P.erythroseptica* in DAC-ELISA but not with extracts of unrelated fungi or a culture of bacterium *Erwinia carotovora*, all of which were either saprophytes or pathogen of potato. Sundaram *et.al.* (1991) raised anti bodies against PAGE separated proteins obtained from an isolate of *Verticillium dahliae.* This could detect 11 different isolates of this pathogen from potato, cotton and soil. It also reacted to a lesser extent with *V.albo-atrum.* They concluded that since the antisera appeared to be specific for *V. dahliae* and *V. alboatrum* and did not cross react with other species of *Verticillium, Fusarium* and *Colletotrichum,* it could be a valuable aid in the detection and identification of *V.dahliae* and *V.albo-atrum.* In studies on the cross reactivity of different isolates of *P.theae* and other fungal genera it was also reported that the antiserum raised against one isolate cross reacted with antigenic extract from other isolates but not from other genera (Chakraborty *et.al.*, 1996). Results of the present study, as well as those of previous authors therefore point to the occurrence of cross reactivities within the isolates of the same species and to some extent within related species but not with unrelated species or genera.

Even though pathogen can be detected in infected tissues rather easily the extent of growth of a pathogen within the leaf tissue cannot be easily quantified. Measurement of lesion size, sporulation or disease rating are often used to quantify the manifestation of disease several days after inoculation (Hoffman and Hill, 1989; Desjardin and Gardner, 1989; Ward *et. al,* 1989; Shaik and Steadman ; 1989). Lesion diameter defines the limits or spread of a colony but yields little information about the mass of hyphae within the colony. Moreover, visible symptoms like lesions do not appear within the first 2-3 days after infection of healthy tissue. Though quantification of plant virus antigens in host tissues by ELISA using samples of antigen of known concentration is now well established (Clark, 1981; Barker and Harrison, 1985), quantitative estimation of mycelia within infected tissues by ELISA has not been widely reported. In the present study quantitative estimation of *G.cingulata* in artificially inoculated leaves from 6h onwards was done by indirect ELISA using anti *G.cingulata* antiserum. Mycelial fresh weight within the leaf tissue of susceptible variety (B 157) increased till 90h of inoculation while in resistant variety (BT-15) it decreased after 78h. Estimation of fungal biomass in infected leaf tissues of 15 varieties revealed that maximum biomass was obtained in the most susceptible variety UPASI-9. Harrison *et.al.* (1990) estimated the amount of *P.infestans* mycelium in potato leaf extracts by comparing the values obtained in ELISA with those for known concentration of *P.infestans* mycelium. It was further reported (Beckman *et.al.*, 1994) that by optimizing the dilution of sample extracts and the dilution of primary anti *P.infestans* antisera, quantification of the biomass of *P.infestans* in zoospore inoculated potato tuber discs could be achieved by 8-18 hours after inoculation. Differences in growth between a virulent and virulent isolates of *P.infestans* on the resistant potato cultivar Kennebec were quantified by 32-48 hours after inoculation.

Detection of cross reactive antigens between *G.cingulata* and tea leaves as well as detection of the fungus within infected tissues was possible by the antiserum raised against whole mycelial antigen of *G.cingulata.* Since antibodies are not raised to all the proteins in the extracts but rather to specific antigenic proteins, in the next step of the present investigation, it was decided to purify the cross reactive antigens from the crude preparation. This was carried out by a series of purification procedures involving ammonium sulphate saturation, DEAE Sephadex chromatography and SDS-PAGE electrophoresis. Ammonium sulphate saturation from the crude mycelial preparation resulted in a number of fraction each of which was then tested by ELISA against anti *G.cingulata* antiserum. Results revealed maximum cross reactivity in the 80-100% SAS fraction. Alba and DeVay (1985) also purified cross reactive antigens from *Phytophthora infestans* by ammonium sulphate fractionation which

was followed by detection in ELISA. They reported that most of the cross reactive antigens were precipitated at 40% SAS.

Since the 80-100% SAS fraction was found to contain the major antigenic proteins the ability of this fraction to raise antibody was further tested. Antiserum raised against this fraction was tested by both immunodiffusion and ELISA against tea leaf antigens. In agar gel double diffusion test precipitation was observed in the homologous reaction as well as in heterologous reaction with antigen of the susceptible variety (TV-18). This antiserum therefore behaved in a similar manner as that raised against whole mycelial preparation. Presence of the antigenic proteins was confirmed in the 80-100% SAS fraction. Chard *et.al* (1985) also raised antiserum against purified mycelial preparation of *Mycena galopus* and compared with the antiserum raised against whole mycelia. Both the antisera were shown to be specific to *M.galopus* by immunodiffusion tests. In this study evaluation of the antiserum raised against 80-100% SAS fraction of *G.cingulata* mycelial antigen was also done by both DAC and DAS-ELISA. CRAs between *G.cingulata* and selected tea varieties was detected in both ELISA formats using this antiserum. This antiserum could also detect *G.cingulata* infection in inoculated tea leaves by both DAC and DAS-ELISA. Results of all the above therefore confirmed the presence of the antigenic proteins in 80-100% SAS fraction since this fraction was effective in raising specific antiserum against *G.cingulata.*

For further prurification, the 80-100% SAS fraction was subjected to DEAE Sephadex chromatography and the proteins were eluted out in three different buffer systems. All the eluates were tested for cross reactivity with anti *G.cingulata* antiserum in ELISA. ELISA results revealed the antigenic proteins to be eluted out in 0.05 M NaCl in 0.05M Tris-HCl buffer. The fractions containing the antigenic proteins were further pooled, concentrated and subjected to SDS-PAGE electrophoresis. Purification of an antigen from *Mycena galopus* by ammonium sulphate precipitation and DEAE cellulose chromatography was reported by Chard *et.al.* (1985). They showed that the antigenic protein as detected by immunodiffusion tests was present in peak III corresponding to the buffer 0.05M-0.25M NaCl in 0.05M Tris-HCl. SDS-PAGE analysis of the antigenic fraction obtained by purification of *G.cingulata* mycelial extract revealed a band corresponding to 10 KDA molecular weight. No other bands were visible.

Purification of the mycelial antigen from *G.cingulata* was further followed by characterization of the cell wall since antisera raised against antigenic preparations from both mycelia as well as cell wall fractions were equally effective in detection of CRAs and pathogen

in tissues. The chemical nature of the mycelial wall extract was determined by SDS-PAGE and ConA-FITC binding both of which confirmed the cell wall to contain glycoproteins. Two glycoprotein bands of molecular weight 50 to 10 KDA were detected in the extract. It is interesting to note that molecular weight of one of the glycoproteins corresponded to the molecular weight of the purified antigenic protein from mycelial preparations. The antisera against both whole mycelial and cell wall preparations were probably raised against similar components. Previous reports on the chemical nature of fungal cell walls also support their glycoprotein nature (Keen and Legrand, 1980; Beissmann, 1992; Ransom *et.al*, 1992).

Host parasite interactions are generally initiated in nature by the fungal spores since they come in contact with the host cell at the first instance. Therefore, conidial wall also plays an important role in recognition phenomenon. Hence, in this study, the conidial wall was also characterized by agglutination test. Responses of conidia to different lectins revealed that the surface components contained mannose and glucose residues. Further, the presence of glycoconjugates containing L-fucose was also established. Studies on the conidial cell wall surface of *F.solani* and *F.oxysporum* by Cristinzio *et.al.* (1988) revealed that the surface contained α-D mannosyl and α-D galactosaminyl residues whereas other species tested such as *F.culmorum F.graminearum, F.moniliformae, F.xylarioides, F. avenaceus* and *F.sambucinum* did not contain these residues in the outermost layer of the conidial wall. Glycoprotein nature of the material released from conidial wall of *Collototrichum graminicola* was established by Mercure *et.al.* (1995) on the basis of studies with FITC conjugated lectins. They further determined glucose and/or mannose to be components of this glycoprotein.

Results of various experiments of this study has established very definitely the importance of cross reactive antigens between host and pathogen in determining the response of the host to pathogen. This has also been supported by the works of several previous workers (DeVay and Adler, 1976; Chakraborty and Purkayastha, 1983; Chakraborty and Saha, 1994b; Chakraborty et.al., 1995). It is also important in studies on host parasite relationship to determine the cellular location of the CRA. For this purpose in this study fluorescence tests were conducted with loosened cells and cross sections of tea leaves as well as mycelia and conidia of *G.cingulata*. Loosened cells were obtained from calli prepared from stem segments of TV-18. Cross sections of tea leaves (TV-18) along with the loosened cells were treated with either anti -*G.cingulata* or anti TV-18 antiserum followed by staining with FITC conjugated anti rabbit globulin specific goat antiserum. Bright fluorescence was observed in both the loosened cells and in the cross sections of tea leaves (TV-18) treated with either of

the two antisera. In homologous reactions within the tea leaf sections fluorescence was distributed throughout the leaf tissues mainly on the epidermal cells and mesophyll tissues. Treatment of leaf sections with *G.cingulata* revealed that the CRA was concentrated mainly around epidermal cells and mesophyll tissues. Treatment of mycelia and conidia of *G.cingulata* with homologous antiserum amd FITC showed a general fluorescence that was more intense on young hyphae and throughout the surface of the conidia. DeVay *et.al.* (1981a) determined the tissue and cellular location of major CRA shared by cotton and *F.oxysporum* f.sp. *vasinfectum*. On the basis of strong fluorescence obtained at the epidermis, cortex, endodermis and xylem tissues, they suggested that the CRA determinants in roots have a general distribution. DeVay *et.al.* (1981b) also used FITC labelled antibodies for races of *P.infestans* to detect the CRA in potato leaf sections. It was also reported by Chakraborty and Saha (1994b) that CRA between tea and *B.carbonum* were mainly present in the hyphal tips and in patch like areas on the conidia, mycelium and mainly around epidermal cells and mesophyll tissues of the leaf. The cellular location of CRA between *P.theae* and tea leaves was also established by Chakraborty *et.al.*, (1995).

Detection of pathogen in host tissues using antibody based immunofluorescent technique has been reported by several previous authors (Warnock, 1973; Hornok and Jagicza, 1973; Reddy and Ananthanarayanan, 1984). Dewey *et.al.* (1984) suggested, on the basis of immunofluorescence studies that chlamydospores, basidiospores and mycelia of *Phaeolus schweinitzii* contained molecules antigenically related to species specific antigens secreted by mycelia grown in liquid culture. They also demonstrated the presence of mycelium and chlamydospores in naturally and artificially infested soil samples, using this technique. *Phytophthora* could also be detected in soil by immunofluorescence antibody teachnique (Watabe, 1990). Different test formats including indirect ELISA, Western blotting, dip stick, dot blot and indirect immunofluorescence was assessed by Wakeham and White (1996) for their potential to detect resting spores of *Plasmodiophora brassicae* in soil. Among the different test formats they found indirect immunofluorescence to be the most rapid and suitable assay for the detection of resting spores of *P.brassicae* in soil.

Studies on the host pathogen interaction between *G.cingulata* and tea leaves mainly on an immunological basis have revealed the major determinants of the resistant or susceptible reactions to be the cross reactive antigens specific for this reaction. Interestingly the antigenic component from both the whole mycelial as well as cell wall preparations of *G.cingulata* appeared to be similar , at least partly. Low molecular weight protein of approximately 10

KDA was purified from the mycelial preparation and the cell wall also had a glycoprotein of similar molecular weight. The observed lack of difference in specificity of the antisera raised against the two antigenic preparations may be explained by the fact that both contained similar proteins. Both proteins and polysaccharide structures have high potential informational content and play important roles in biological recognition phenomena. Thus, undoubtedly these have very important roles to play in host pathogen interactions and success or failure of infections depend to large extent on these structures. Other than the establishment of cross reactive antigens between *G.cingulata* and tea leaves, a major outcome of the present study has been the detection of brown blight infection in tea leaves at very early stages of infection and at very low amounts. Quantification of the mycelial growth in leaf tissues even before [illegible] visible symptoms have appeared i another important achievement. Such early detection of disease is of extreme importance where conventional techniques may fail to detect infection or infections can be detected only after appearance of well defined symptoms. This can be of great use in the area of disease management and control where it would be possible to take preventive measures if disease can be detected sufficiently early, before the appearence of visible symptoms. This would lead to minimisation of economic loss due to desease.