Discussion

Plant pathogen interaction is a highly complex series of events triggerred by the deposition of pathogen inoculum on the host. This is followed by the invasion of host tissue by the pathogen which inturn initiates the complex and variable developmental pattern between host and parasite which continues throughout their course of coexistence. Differences in physiological responses and morphological structures of various host genotype affect their susceptibility or resistance in invasions and its consequences while similar variation in pathogens influence their growth rate and virulence (Loomis and Adams, 1983). The host genotype or the biochemical make up of the host alone can not determine the course of disease development which is also dependent to a great degree on the environmental conditions prevailing in a particular area. Thus, in nature complex series of interaction between the environment, the host and the pathogen, finally determines whether the pathogen will be successful in establishing disease or the host will be successful in warding off the pathogen.

At the beginning of this study, nine varieties of tea were screened for resistance against *Glomerella cingulata* under laboratory conditions. TV-18 was most susceptible followed by TV-23, and TV-26 the most resistant followed by TV-29 under both detached and cutshoot inoculations.

Disease incidence under natural conditions in the field also revealed TV-23 and TV-18 to be most susceptible and TV-26 to be most resistant. Result of the present study clearly indicate that tea varieties exhibited differential resistance to *G. cingulata*. Such result have been obtained by previous workers. Gupta *et al.* (1996) screened several sorghum germplasm (Multicut) against important foliar diseases i.e. downy mildew (*Peronosclerospora sorghi*), sooty strip (*Ramulispora sorghicola*), zonate leaf spot (*Gloeocercospora sorghi), grey leaf spot (Cercospora sorghi*) and anthracnose (*Colletotrichum graminicola*). They reported that during 1993 five genotypes i.e. IS 3266, 32407, 70742, 71664 and 6018 exhibited resistance to all five diseases and only one i.e. IS 3266 showed such resistance in 1994. Evaluation of carnation germplasm for resistance against *Alternaria* blight showed Yellow Dusty, Harvest Moon, Leena and shocking pink to be highly susceptible to *Alternaria* blight and scania to be least susceptible (Meeta *et al.* 1996). Resistant linseed genotype against *Alternaria* blight and powdery mildew were also screened by Kaur and Lenka (1998). No genotype was fully resistant while a number exhibited moderate resistance or susceptibility. Chambol and Subhra were highly susceptible to *Alternaria* blight and LCK 9325 to powdery mildew. Kumar *et al.* (1999) screened 128 varieties/ lines against *Drechslera graminia* and obtained 53 highly resistant, 11 resistant, 6 moderately resistant, 11 moderately susceptible, 6 susceptible and rest of the varieties highly susceptible. Studies by Singh (1999) also revealed that different sugarcane varieties exhibited large variation in their disease reaction to whip smut caused by *ustilago scitaminaea.* Out of twenty five varieties, four were found to be resistant and there highly resistant. Sharma and Badiyala (1998) screened 21 Cultivars of mango for susceptibility to *Collectorichum gloeosporoidis* during different seasons. They reported that none of the cultivars and seedling selection of mango was resistant to anthracnose disease. Higher numbers of spots (62.66) developed on cultivar Amrapali followed in order by Totapari, Lala Da Amb, Safeda and Mallika and these five cultivars were rated as highly susceptible whereas eight others were moderately susceptible. Maximum disease developed on leaves during rainy season followed by spring and minimum in post rainy inoculated leaves. These results are in conformity with those of the present study where maximum disease was obtained during rainy season followed by summer and autumn, both under artificial inoculation and natural conditions. Besides different, seasons, disease development was also affected by other factors like age of culture of inoculum, spore concentration (inoculum density) and available light. Ten days old culture with a concentration of $1.6x10^5$ spore / ml under diffused light conditions were most favourable for brown blight development. Singh and Thapliyal (1998) reported that the pathogenic potential of *Sclerotium rolfsli* causing seedling rot of soybean increased with increased inoculum density. Soybean cultivar PK 327 was slightly tolerant and Bragg highly susceptible to pre-and post emergence rot.

The habitat of pathogen which invades the aerial parts of the plants is immediately and profoundly influenced by weather. These pathogens usually reproduce abundantely and with the onset of favourable conditions spread rapidly from a minimum amount of initial inoculum (Rotem, 1978). The ability of a pathogen to survive during periods of adverse conditions enables it to carry over from one season to another. Atmospheric parameters influencing disease development are usually temperature, relative humidity, rainfall, plant density etc. In the present investigation relationship between occurrence of brown blight disease and weather factors like temperature, humidity, rainfall and sunshine hours were determined in six varieties for three consecutive years. Positive correlation of disease intensity was obtained with minimum temperature, relative humidity and rainfall and negative correlation with hours of sunshine. Most favourable condition for brown blight development appeared to be a humid atmosphere with a minimum temperature of 24^{0}C, heavy rainfall and lesser hour of sunshine. Such weather conditions are generally present. during rainy season and hence disease development was maximum in rainy season. Several previous workers have worked extensively on the relation of weather conditions to disease development. Rolando *et al.* (1989) reported that the level of infection of *Eucalyptus* by *Puccinia psidii* varied with temperature, leaf wetness period and photo period. Higher disease intensity was observed at 20-25^{0}C after 24 hours of wetness of leaf surface and disease was inversely correlated with leaf exposure during incubation period. Detailed analysis of the effect of rainfall variable on the epidemiology of *Phytophthora* blight of pepper was carried out by Bowers *et al.* (1990). They obtained largest absolute direct effect by the cumulative amount of rainfall while the cumulative number of days with rainfall, the cumulative daily average temperature and chronological time had far lesser effect. Temperature and duration of wetness period also influenced the development of shot hole disease on almond leaves caused by *Willsonomyes carpolophilus* (Shaw *et al.* 1990). A functional relationship between anthracnose of bottle gourd and meteorological factor was established by Gandhi *et al.* (1997).

Temperature and relative humidity showed significant effect on disease epidemiology. Rainfall leading to high R.H and less sunshine hours had an important role in disease development. These results are in confirmility with those of the present study. In the present investigation disease was found to occur during most part of the year other than the winter months (November, December, January). Extreme cold condition was not suitable for disease development.

Age of leaves was also seen to be correlated with the degree of disease intensity. Under laboratory conditions younger leaves were more susceptible to disease development than the older leaves. However when survey of disease was conducted in the field it was observed that disease symptoms were visible only on the lower leaves. The apparently contradictory observation on field and laboratory condition can be explained by the fact that in the field the young tea leaves are continuously plucked and hence the time period necessary for inoculum deposition and spore germination is hardly available. The older leaves which remain for longer periods get wounded sooner or later following which the pathogen enter and disease development occurs. This is because the brown blight pathogen can normally gain entry into the leaf only through wounds (Bertus, 1974; Dickens and Cook, 1989). Dubey (1997) reported that ground nut plants were susceptible to *Thanotophoras cucumaris* at early stage of growth though susceptibility increased with increasing plant age upto 30 days. Maximum disease development was also favoured by a temperature of 25-28^{0}C and relative humidity of 98-100%. Atmospheric temperature and relative humidity were also shown to play a determining role in the initiation and subsequent development of powdery mildew of mango (Verma and Kaur, 1998); They observed that minimum atmospheric temperature plays more prominent role in the initiation of fresh infection besides cloudiness along with muggy and warm weather. Initiation of red rot infection of sugarcane was correlated to climate factors (Kumar *et al.* 1998). Red rot initiation was significantly and negatively correlated with maximum temperature while the reasons of minimum temperature was non significant. Contribution of the rest of the weather variables in red rot initiation was non significant. Roger *et at.* (1999 a and b) conducted detailed study to determine the effects of temperature and moisture and interrupted wet periods on the development of *Mycosphae rella pinodes* on pea. Disease severity and the number of pycnia formed on leaves increased with temperature from 5-20^{0}C and then decreased between 20-30^{0}C. Pycinidiosores were reported to survive in dry periods up to 21 days of inoculation with the infection capacity being restored following re-wetting. Results of the present study along with those of a number of previous workers establish the definite role of environmental factors on disease development as well as initiation of disease.

Climate influences disease development by its effect on the plants as well as on the pathogens (Coakley;1998). Plant factors associated with disease included plants own resistance and susceptibility, age of plant, plant density etc. The growth of the pathogen in the host is dependent to a large degree on these factors as well as on environmental factors. Fungal plant pathogens invade host plant cells with a variety of specialised infection structures of which pressorium is, in many cases, the most important structure formed in preparation of host colonization (Hoch and Staples, 1987). It must be positioned at appropriate site on the host in the most advantage way for subsequent infection to occur and it must be able to withstand adverse environmental conditions. For *G. cingulata* which is able to infect only through wounds, the positioning of appressorium assumes great significance. Ando and Hamaya (1986) reported that the anthracnocse fungus *Gloeosporium thea sinesis* could infect tea only through the trichomes of young leaves.

In the present study, therefore a series of experiments were conducted to determine the various factors affecting spore germination, appressoria formation and mycelial growth of *G. cingulata invitro.* Spore germination, appresioria formation and myceliel growth were affected by different factors like temperature period of incubation, pH, spore concentration, source of nutrient etc. Spore germination and appresoria formation of *G. cingulata* were maximum at 25^{0}C, pH 5.5, diffused light and after 24 hours of incubation using a spore concentration of 1.6x10^5 spores / ml. An incubation period of 10-12 days at pH 6.5-7 with maltose and KNO_3 as carbon and nitrogen sources respectively were optimum for mycelial growth.

Studies of previous authors on various pathogenic fungi established the optimum conditions for spore germination and mycelial growth of these. Jordan *et al.* (1990) found isolates of *Cladosporium allii* and *C. allii cepae* to grow best at 20^{0}C and 15-20^{0}C respectively on 2.5% malt extract agar. Both species tolerated a wide range of pH with maximum sporulation at 5-8.5. Al least 8h. of darkness was necessary for optimum germination of both fungi. Conidia of *Cercospora* species were found to germinate within 2h and 100% germination was recorded by Raghuram and Mallavia (1996) within 8h of incubation. They reported that the conidia of *Pseudo cercospora* species took relatively longer time to germinate than *Cercospora* species. Species of both the above fungi sporulated at r.h. levels between 66-100%. The role of temperature and relative humidity on spore formation of *Uncinula necator,* the causal agent of powdery mildew of grape was determined by Chavan *et al.* (1995). Temperature in the range of 22.2-30.1^{0}C and R.H greater than 57.4% favoured spore production by the pathogen . Among ten carbon sources tested for their effect on growth and sporulation of *Fusarium sp.* causing sheath rot of rice, sucrose, xylose and fructose supported the maximum growth of *F. graminearum, F. moniliforme* and *F. avenaceum* respectively. Sucrose induced maximum sporulation in *F. avenaceum* (Singh and Devi,1996). L-histidine, D. L-asparatic acid and D, L-alanine supports maximum growth of *F. avenaccum, F. moniliforme* and *F. graminearum* respectively. *Colletotrichum truncatum* was reported to have an optimum temperature of 20^{0}C for germination with 3h light followed by 9 h dark (Kaushal *et al.* 1998). Ahmed and Mir (1998) reported that all carbon and nitrogen compounds tested supported vegetative growth of *Willsonomyces carpophyllus* though maltose proved best carbon source followed by mannitol, glucose and suerose. Similar results have also been obtained in the present study where maltose was the best carbon source.

W. carpohyllus also exhibited maximum mycelial growth in medium containing asparagine as the nitrogen source. The above studies clearly point out that the fungi differ greatly in their requirements for growth and sporulation. Not only do the optima for temperature, pH etc. vary but the different fungi also utilize nutrients differently. A particular carbon or nitrogen source which may support good growth of one fungus may not be suitable for another.

Other than environmental conditions, in plants, their biochemical make up is responsible for the particular response shown by plant to a pathogen. The biochemical constituents are again influenced by existing environmental conditions. A particular biochemical component may vary according to the variety of the plant, age of the plant / plant parts, season etc. Accumulation of phenols in plants is one of the most well studied aspect with respect to host pathogen interaction. Many of the phenols in plants occur constitutively and are associated with resistance (Cole, 1984; Baker *et al.* 1989, Chakrarborty *et al.* 1995). In the present study accumulation of phenols in different tea varieties has been thoroughly investigated. No significant correlation between phenol content and resistance and susceptibility of tea leaves to *G. cingulata* could be discerned. Maximum phenol accumulation however was obtained in rainy season. Nosolillo *et al.*(1989) reported that accumulation of phenolic substance in pine seedlings was pronounced throughout October and November. Total phenolics showed a gradual increase over the season. In the present study the phenolic contents were seen to be maximum in the young leaves. Inoculation with *G. cingulata* resulted in an increased accumulation of both total and Orthodihydroxy phenols in resistant genotype of pea was reported by Sharma *et al.* (1996). Sindhan and Parashar (1996) also reported that the total phenol was higher in the resistant cultivars i relation to susceptible ones and that accumulation of phenols was greater in resistant varieties of ground nut infected by early and late leaf spot pathogen than in susceptible cultivars. However, Mitter *et al.* (1997) reported that though chickpea genotypes resistant to gray mould had higher total phenols, the phenols decreased after inoculation by *B. cinerea,* Gularia *et al.* (1998) also reported that the resistant cultivars of pea had higher phenol contents but inoculation with *Erysiphe polygonii* led to a marked increase in the O-dihydroxy phenol only. In most of the studies therefore resistant cultivars have been shown to have higher phenol content than the susceptible ones, which increased following infection by a pathogen. In the present study, though no significant difference in resistant and susceptible varieties of tea could be discerned, a greater accumulation of phenols following inoculation in the resistant varieties was seen, which is in confirmily with the reports of the previous workers. Besides total phenol, higher levels of diffusible phenolic compounds was obtained in the resistant cultivar. Concentration of diffusible phenol was 2-3 times greater following inoculation of tea leaves. It seems probable that rapid accumulation of phenol at the infection site may result in the effective isolation of pathogen or nonpathogen at the original site of ingress. Since polyphenols are the major constituents of tea leaves, their involvement in the defense mechanism can be expected. It has been reported by Chakraborty *et al.* (1995) that among several phenolic detected in tea leaves catechin was antifungal in nature.

Chlorophyll is undoubdtedly the most important plant pigment since it controls photosynthetic activity of a plant and thereby determines the plant's productivity. Any factor affecting chlorophyll accumulation is therefore of prime importance to the plant. In the present study, chlorophyll accumulation was considered to be one of the biochemical parameters to be investigated and changes, in chlorophyll content due to varietial difference, age of the leaves and following infection by *G. cingulata* were determined. Chlorophyll content varied among the different varieties tested and was higher in the older leaves. Infection with *G. cingulata* resulted in a reduction in chlorophyll content which was quite marked. De Silva and Sivapalan (1982) also studied the chlorophyll content of different tea clones and reported differences among them. Maximum chlorophyll accumulation was obtained by them in the third leaf. They suggested that the chlorophyll content of tea plants varied with shade, season, geographical location and clones. Wide variation in chlorophyll a, b and total chlorophyll were observed among 36 T.R.A. Garden series clones by Bera *et al.* (1997). They grouped these clones into 3 categories - low, medium and high, according to their pigment contents. Studies on effect of infection by pathogen on chlorophyll content have been done by previous workers who also reported a reduction in chlorophyll accumulation following infection (Bhavani *et al.* 1998, Sutha *et al.* 1998, Singh *et al.* 1998). Thus these results are in conformity with those of the present study. The reduction in chlorophyll contents might be due to stimulating of enzyme chlorophyllase which degraded chlorophyll (Kaur and Deshmukh, 1980) or inhibition of chlorophyll synthesis caused by the pathogen (White and Brakke, 1983).

Epicuticular wax is the outermost layer in the leaves of the plant and plays a fundamental role as a barrier between the leaf and the environment-most specifically attack by the pathogens. The amount of wax on leaf surface will also be one of the factors determining a plant's resistance or susceptibility. Among the varieties tested maximum ECW content was obtained in resistant cultivar and the older leaves had higher ECW than the younger leaves. Mohammad *et al.* (1986) also reported that the wax content was higher in drought resistant tea clone them in the susceptible ones. Very significant differences were also reported in ECW content among sorghum cultivars by Ebercon *et al.* (1977). In the present investigation it was also observed that infected leaves had significantly lower ECW content them the healthy ones. It seems provable that during the course of infection the fungus was successful in degrading epicuticular wax. In a study conducted on significance of epicuticular wax in specificity of blast fungus to rice varieties by Kumar and Sridhar (1987), they found that ECW from resistant cultivar inhibited appressoria formation of *Pyricularia oryzae* while ECW of susceptible cultivar stimulated appressoria formation. They suggested that resistance or susceptibility may in part be governed by this mechanism.

Results of present detailed study on brown blight disease of tea caused by *G. cingulata* have shown that growth of pathogen and its subsequent establishment in the host is governed by a number of environmental factors as well as by genetic make up of each variety causing it to be either susceptible or resistant. Environmental factors are very important as they not only influence the growth of the pathogen and disease development but also influence the growth of the host as well as biochemical components. Such changes of these biochemical components also are involved in regulating the plants response to the pathogen. Thus it can be generalised that the final appearance of disease on host is dependent on a multitude of factors - both external and internal.