Summary

(1) A review of literature pertaining to this investigation has been presented which deals mainly with serological cross reactivity between host and pathogen, detection of plant pathogenic fungi and serological cross reactivity among fungal species.

(2) Materials used in this investigation and experimental procedures followed have been discussed in detail.

(3) Pathogenicity of *Glomerella cingulata* was tested on 37 varieties of tea (13 Tocklai varieties 15 Darjeeling varieties and 9 UPASI varieties). UPASI 9, TV-18 and B-157 were found to be the most susceptible while CP-1 and BT-15 were resistant.

(4) Maximum growth of *G.cingulata* occurred after 10 days of incubation and at a pH of 6.5.

(5) Maltose was the most effective carbon source and potassium nitrate the most effective nitrogen source for optimum growth of *G.cingulata.*

(6) Antisera were raised against antigen preparations from mycelia and cell wall of *G.cingulata* (isolate GC-1), tea leaves (TV-18 and CP-1) and *F.oxysporum* (non-pathogen of tea).

(7) In agar gal double difusion tests anti *G.cingulata* antiserum was cross reacted with leaf antigens of all 37 tea varieties, 3 non host species and a non pathogen of tea. Strong precipitin reaction occurred in homologous reactions and in reactions involving susceptible varieties while, no precipitation occurred with resistant varieties, non hosts or non pathogen.

(8) Reciprocal cross reaction was performed with antisera of TV-18 (susceptible variety) and CP-1 (resistant variety) and leaf antigens of host and non host as well as mycelial antigens of pathogen and non pathogen. Precipitation was observed in reactions of most of the tea leaf antigens. Antiserum of TV-18 reacted strongly with antigen of *G.cingulata* but antiserum of CP-1 did not. No cross reactions were observed with non host species or non pathogen.

(9) Immunoelectrophoretic tests revealed cross reactivity of anti *G.cingulata* antiserum with its isolates, and susceptible tea varieties. No precipitin arcs were discerned with resistant varieties, non hosts or non pathogen.

(10) Reciprocal reaction in immunoelectrophoresis also confirmed cross reactivity in susceptible reacftions but not in resistant reactions.

(11) Rocket immunoelectrophoresis using anti *G.cingulata* antiserum showed 3 immunoprecipitin lines in homologous reactions and a single line for the susceptible reaction.

(12) All the antisera raised were purified by ammonium sulphate precipitation and DEAE Sephadex chromatography. IgG, obtained in each case was used for ELISA tests.

(13) Optimum conditions for ELISA reactions were determined. An antiserum dilution of 1:250 and an enzyme (alkaline phosphatase) dilution of 1:10,000 were optimum for antisera raised against both mycelial and cell wall preparations of *G.cingulata* in homologous reactions.

(14) Both the antisera detected homologous antigens upto a concentration of 25 ng/ml .

(15) Comparison of ELISA reactivities of all the 37 varieties of tea with anti *G.cingulata* antiserum revealed highest absorbance in the variety UPASI-9 and lowest in BT-15.

(16) In reactions with antiserum raised against cell wall preparations also, maximum absorbance was obtained for UPASI 9 and minimum for BT-15. Absorbance values in this case were generally higher than for reactions with mycelial antiserum.

(17) All the isolates of *G.cingulata* tested showed high absorbance values with antiserum raised against one of the isolates (GC-1).

(18) Absorbance values for reactions with non hosts and non pathogens were low.

(19) Indirect ELISA reactivity of antigens, with anti *G.cingulata* antiserum (mycelia and cell wall) from tea leaves of different ages did not show any significant difference.

(20) Reciprocal cross reactions with antisera raised against TV-18 and CP-1 showed high absorbance values in susceptible reactions and low values in resistant reactions as well as reactions with non host and non pathogen.

(21) Antiserum raised against a non pathogen did not react with any of the antigens as evidenced by very low absorbance values in ELISA.

(22) Direct ELISA (DAS) of anti *G.cingulata* antiserum with antigens of different tea varieties, non hosts and non pathogen revealed maximum absorbance in susceptible reactions.

(23) *G.cingulata* was detected in artificially inoculated leaves of all tea varieties on the basis of significantly higher absorbance values in ELISA of inoculated extracts as compared to healthy extracts, using anti *G.cingulata* antiserum. Similarly, natural infection could also be detected in ELISA.

(24) *G.cingulata* was detected as early as 6h after inoculation in a susceptible variety (B 157) and after 30h in a resistant variety (BT-15) using anti *G.cingulata* antiserum in ELISA reactions.

(25) Detection was done after 6 and 18h of inoculation respectively in the susceptible and resistant varieties by ELISA using antiserum raised against cell wall preparation of *G.cingulata.*

(26) In ELISA, infection could be detected till a concentration of 1 μg/ml of infected leaf extract on the basis of significantly higher absorbance values of infected extracts as compared to healthy extracts.

(27) ELISA values extracts of tea leaves inoculated with *P.theae* or *C.invisum* were low when tested against anti *G.cintulata* antiserum.

(28) The occurrence of *G.cingulata* as the predominant pathogen in a mixed natural infection was established by competition ELISA using antisera of both *G.cingulata* and *P.theae.*

(29) Antiserum of *G.cingulata* (isolate GC-1) showed cross reactivity in ELISA with antigens of 8 other isolates but not with antigens of different species of *Colletotrichum, P.theae* or *C.invisum.*

(30) Estimation of fungal biomass of *G.cingulata* was done by ELISA from infected tissues after different hours of inoculation. On the basis of standard curve of mycelial fresh wt. versus absorbance in ELISA maximum mycelial fresh weight was obtained in the susceptible variety after 96h of inoculation. Of the different varieties tested, maximum growth was estimated to be in the susceptible varieties.

(31) Purification of antigenic protein of *G.cingulata* from the crude mycelial extract by ammonium sulphate saturation and DEAE Sephadex chromatography was done and detection was done by ELISA. SDS-PAGE electrophoresis of the purified protein revealed one band at approximately 10 KDa.

(32) Partially purified (80-100% SAS fraction) antigenic preparation was used for raising antiserum and this antiserum was tested by immunodiffusion tests, DAC ELISA and DAS ELISA with homologous and heterologous antigens. This antiserum also showed higher reactivity in the susceptible reactions as compared to resistant reaction.

(33) The antiserum raised to 80-100% SAS-fraction could also detect infections by ELISA.

(34) Characterization of the cell wall of *G.cingulata* by ConA-FITC binding and SDS-PAGE electrophoresis revealed the glycoprotein nature, with 2 bands of 50 KDa and 10 KDa molecular weights.

(35) Agglutination test of conidia of *G.cingulata* with different lectins revealed strong agglutination with ConA followed by UAE I. The presence of glycoconjugates containing glucose and/or mannose residues as well as L-fucose was confirmed on the outer surface of the conidial wall.

(36) Callus was induced from stem segments of TV-18 and from this callus loosened cells were obtained. Immunofluorescence studies of these cells with homologous antiserum or anti *G.cingulata* antiserum revealed bright fluorescence after treatment with FITC.

(37) Cross sections of tea leaves (TV-18) treated with homologous antiserum and then with FITC, developed a bright fluorescence throughout, which was concentrated mainly in epidermal cells and mesophyll tissues. Cross sections of the leaf exhibited autofluorescence also.

(38) Treatment of cross sections of tea leaf with anti *G.cingulata* antiserum and FITC revealed bright fluorescence mainly around epidermal cells and mesophyll tissues.

(39) Mycelia and conidia of *G.cingulata* when treated with homologous antiserum or anti TV-18 antiserum and then with FITC revealed bright fluorescence mainly on young hyphae and throughout the surface of conidia.

(40) The implications of the results have been discussed.