BIOLOGY AND PATHOGENICITY OF *PHYTOPHTHORA MEADII* ISOLATES CAUSING LEAF FALL OF RUBBER AND SOME HOST PATHOGEN FACTORS INVOLVED IN INFECTIONS

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A thesis submitted to the
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University of Colombo
For the Degree of
Doctor of Philosophy

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2002

ABSTRACT

Phytophthora leaf fall is one of the main diseases in rubber (*Hevea brasiliensis* Muell. Arg.) plantations. The disease has to be managed by chemical application, which is not economical. The best remedy is breeding highly resistant genotypes (clones). The understanding of factors involved in host pathogen interactions leading to host resistance was the main concern in the study. The causal agent of the disease, *Phytophthora* meadii McRae was isolated from infected rubber genotypes (clones), which were either resistant or susceptible to *Phytophthora* leaf disease (*P*ld). Isolates were differentiated based on their growth, morphology and degree of virulence. Growth and virulence varied among the isolates obtained from the same and/or from different clones. Some isolates obtained from *P*ld susceptible clones (PB86 or RRIC121) were highly virulent, compared to the isolates obtained from resistant clones. Highly virulent isolates produced more sporangia on lima bean agar when incubated at 27 ± 2° C.

The virulence of seven *Pm* isolates had relation with the *in vitro* secretion of cell wall degrading enzymes such as polygalacturonase (PG). Five isolates produced PG having molecular weights of 62-85 kDa. The most virulent isolate MAD86 (IMI 385259) produced two forms of PG including a form having a lower molecular weight (48 or 21 kDa). The low molecular weight PG's were produced only by MAD86 and the isolate show the next highest virulence PE102b. No pectin lyase (PL) was produced by any of the isolates studied. Further, neither PG nor PL was detected in infected rubber petiole tissues.

Antifungal compounds available in rubber petioles are vital to suppress the *Pm* establishment during infections. As one aspect of the study, petiole extracts were obtained in 50% boiling ethanol and tests revealed that petioles of *P*ld resistant clones had more pre-infected antifungal compounds than that of petioles of susceptible clones. Pe obtained from healthy clones inhibited the germination of *Pm* (IMI385259) zoospores and growth in pea broth. Pe of resistant clones caused significantly high inhibition of germination and growth. The compounds related to the inhibition were subjected to phytochemical screening and detected as flavonoids, leucoanthacyanins, sterols or triterpines, tannins or polyphenols, steroids or terpinoides.

It was of vital importance to analyze the post-infectional antifungal compounds that might be responsible for suppressing the *Pm* infection in a clone, which is resistant to *P*ld. Methanol:methylenechloride (1:1 v/v) extraction of dried healthy petioles of RRIC100 (resistant to *P*ld) contained vanillin (3-hydroxy-4-methoxy benzaldehyde), a highly fungi-toxic phenolic compound (LD_{50} = 0.45 μMol ml^{-1}) whereas *Pm*-infected petioles contained phenolics such as stillbenes or flavonoids. Extracts of healthy petioles of PB86 (susceptible to *P*ld) contained triterpines or flavonoids whereas *Pm*-infected petioles had hydroxycoumerins or stillbenes.

Latex serum was also taken into account as a substance available in rubber petiole that might have some impacts on pathogenic infections. However, latex serum from resistant clones significantly promoted zoospore germination, while serum from susceptible clones either had no effect or significantly reduced germination.

Post-infectional phenolic compounds are produced by host's phenylpropanoid pathway. Phenylalanine ammonia-lyase (PAL) activity is the key factor in the production of phenolics. Significantly higher PAL activity was always observed in *Pm*-infected resistant than in susceptible clones. Production of pathogenesis-related (PR) proteins was also considered as an important defense mechanism in host tissues, and such proteins were present in resistant clones. However, only three PR protein bands were observed in the examined clones.

Exudation of anti-fungal compounds from the petiole surface upon infection was another important aspect of the study. *P*ld resistant clones exuded significantly more phenolics and proteineous compounds than susceptible clones.

Pm infection was also considered to be affected by phylloplane microflora and the surface ultra-structure of petioles. Petiole surfaces of the clones RRIC100 or PB86 were investigated. Bacterial species antagonistic to *Pm* were isolated only from the susceptible surface. *Penicillium* and *Pestalotia* spp. were isolated from both clones, while *Fusarium* and *Botriodiplodia* spp. were isolated from the susceptible petioles. *Trichoderma* species were isolated only from the resistant petioles. The number of vesicle openings on the surface of susceptible petioles surface was higher than on the resistant clone surface. Petiole surface microflora of both clones affected the incidence of *P. meadii* infection on petioles.

As significant characteristics of *P*ld resistant *H. brasiliensis* clones, higher PAL activity, rapidly accumulation or exudation of higher phenolic compounds to petiole surface, presence of highly antagonistic microbes on the phylloplane microflora were observed. The possibilities were explored for using those criteria for identifying new *P*ld resistant clones in breeding programs.