BIOLOGY AND PATHOLOGY OF THE CLOVE ISOLATE OF *Cylindrocladium quinqueseptatum* BOEDIJN & REITSMA

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SUMMARY

Cylindrocladium quinqueseptatum Boedijn & Reitsma causes seedling blight and extensive defoliation on a wide variety of plants and the fungus is widely distributed in the humid tropics. In Sri Lanka, *C. quinqueseptatum* is a serious pathogen of *Eugenia caryophyllata* and is identified as a potential pathogen of *Hevea brasiliensis*.

Cultural characteristics and reproductive morphology of the clove isolate of *C. quinqueseptatum* (IMI 342173) were shown to be similar to *C. quinqueseptatum* isolates studied in different parts of the world. Dimensions and photomicrographs on morphology of IMI 342173 (Rt) are also provided.

Some isolates collected from different clove growing localities in Sri Lanka showed a great deal of variation in their morphology. Subsequent studies showed that a distinct variation exists among *C. quinqueseptatum* isolates and differences in (a) morphology, (b) sporulation on CDA and (c) growth at room temperature are significant and provide a very useful characters to separate them.

Lesions produced on *E. caryophyllata* as well as on twenty one *H. brasiliensis* clones by the different isolates following inoculation varied in size significantly showing differences in virulence among the isolates. Isolate, Aw was the most virulent while Rw isolate caused the smallest lesions consistently on *Hevea* clones indicating it's mild nature. A considerable variation in susceptibility existed among the *Hevea* clones grown in the Eastern Hemisphere. Clones PB 28/59, HP 74-181, Tjir 1, RRIC 121, RRIC 45, RRIC 36, RRIC 130, RRIC 110, RRIM 712, RRIM 600 were the most susceptible and IAN 873, AV 1373, RRIC 102 and IAN 717 were among the least susceptible clones.

The fungus sporulated freely when grown on artificial media viz. Czapek Dox Agar and Lima Bean Agar, both under the normal light and dark regime and under continuous dark. Spore production occurred between 20°C and 35°C with an optimum at 30°C. Spore productivity was highest in the Aw isolate.

The maximum spore germination observed was around 90% after 5 h incubation as wet smears on *Hevea* leaves. A period of 10 min exposure of spores as wet smears to UV (253.7nm) inactivated the spores significantly and 40 min exposure was detrimental. The most critical factor which influenced the spore viability and germination was the humidity. Free water or a film of water (resulted as dew formation at 100% RH) was found to be essential for spore germination. With regard to the lesion production on leaves, lesions with a reasonable size were produced only at 100% humidity. Size of the lesions at 96% humidity was negligible and no lesions were produced at 91% RH. The temperature also greatly influenced the spore viability and germination. Spore germination occurred above 10°C and below 35°C. Thus it was shown that the optimum conditions for germination of spores are those conditions present in the rubber growing districts of Sri Lanka during the South-West monsoon period.

All isolates were found to be capable of secreting toxic substances to the growing medium and this toxin was proved to be thermostable (up to 100°C) and host specific. A technique was also developed to partially purify the toxin secreted by *C. quinqueseptatum*. The type and the size of the lesions produced on *Eugenia* and different *Hevea* clones by the crude toxin of different isolates varied markedly. Isolate Rt produced the largest lesions overall on *Hevea* indicating its aggressive nature in toxin production. Isolate Kp was also found to be very active while Aw and Rw showed only a mild reaction. Three main clusters of clones were distinguished through cluster analysis indicating the marked variation of *Hevea* clones grown in the Eastern Hemisphere in sensitivity to the crude toxin.

None of the *C. quinqueseptatum* isolates secreted polygalacturonase (PG) (except Kp isolate) or pectin lyase (PL) in culture. Isolate Kp showed mild PG activity. The extracts of the clove and rubber leaf tissues inoculated even with Kp isolate did not reveal any PG or PL activity. Our investigations, however, failed to show the involvement of an inhibitor of PG. All isolates of *C. quinqueseptatum* secreted cellulases viz. cellobiase and β-glucosidase in culture. A marked increase in activity of cellobiase was detected on rubber leaf on third day following the infection and activity of β-glucosidase, an inherent enzyme of *Hevea* leaves increased markedly following infection. Considering the above observations and available literature it is proposed that toxins play a vital role in initial stages of infection and development of symptoms while latter stages of interaction may be due to the activity of cellulolytic enzymes.

A total of sixteen fungicides were screened against *C. quinqueseptatum* employing three screening techniques; conidial germination test (CGT), poisoned food technique (PFT), and soil fungicide screening test (SFST). Though there were eleven fungicides effective in CGT, this number was reduced to five in SFST. However, only four viz. benomyl, mancozeb, metalaxyl 8% + mancozeb 64% and oxadixyl 10% + mancozeb 56% were identified as potential fungicides in the management of *C. quinqueseptatum*. It was shown that, for a pathogen producing microsclerotia, SFST is the most appropriate test. Further, the incubation period after treatment was of vital importance in CGT as low incubation periods provide misleading information on the efficacy of fungicides. In addition to chemical control, possibility of the development of a successful breeding programme to produce disease resistant *Hevea* clones, using observations reported on clonal susceptibility, is discussed.