Jl. Rubb. Res. Inst. Sri Lanka (1982) **60**, *41–46*

HOST-PARASITE RELATIONSHIPS OF COLLETOTRICHUM LEAF DISEASE IN RUBBER

By

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SUMMARY

Conidia of *Colletotrichum gloeosporioides* germinated equally well on both susceptible and tolerant *Hevea* clones. Initially, there was no difference in germ tube growth between clones, but at 24 h after inoculation clones RRIC 52 and PB 86 showed the maximum and minimum growth, respectively. Appressoria were observed 6 h after inoculation, with clone RRIC 52 producing significantly more appressoria than PB 86. They were broader than the conidium and were frequently observed between anticlinal walls of adjoining epidermal cells. The fungus gained entry into the host about 9 h after inoculation and grew inter and intra cellularly. Acervuli were produced 72 h after inoculation.

INTRODUCTION

Colletotrichum gloeosporioides Penz., (*Gloeosporium alborubrum* Petch), the causal organism of *Colletotrichum* leaf disease (CLD) of the para rubber tree (*Hevea brasiliensis* Muell. Arg.), was first recorded in Sri Lanka in 1905 (Petch, 1921). It affects immature leaves of nursery plants, budded stumps and those formed after annual wintering, causing premature defoliation, referred to as secondary leaf fall (SLF). The disease is present throughout the year but is most pronounced during wet weather when dieback of shoots occurs due to repeated defoliation (Wimalajeewa, 1963). Detailed studies have been done on the biology (Wimalajeewa, 1965; Wastie, 1972) and control of the disease (Peries and Wimalajeewa, 1970 ; Wastie, 1972; Liyanage and Dharmaratne, 1975). Although field resistance of several clones is known (Liyanage, 1976; 1977), there is little or no information on host-parasite interactions. This paper reports on the preliminary findings of the pre-penetration behaviour of *C. gloeosporioides* on susceptible and tolerant *Hevea* clones and on the post penetration behaviour in a susceptible clone.

MATERIALS AND METHODS

Isolation: Several infected leaves of the clone PB 86 were obtained from a 3-year-old budwood nursery at Dartonfield Estate. They were incubated in moist chambers in the laboratory at room temperature (RT) 28 ± 2^{0}C. A pink mass of spores of *C. gloeosporioides* was produced within 2 days and the fungus was isolated by plating spore samples on 3.9% Difco potato dextrose agar (PDA). The cultures were grown at RT

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until sporulation occurred. Conidia were then scraped into sterile distilled water and filtered through three layers of muslin cloth. A single spore culture, obtained by dilution plate method, was used for all subsequent studies.

Inoculum preparation: A spore suspension for inoculation experiments was prepared by adding 10 ml sterile distilled water to a sporulating culture. The spores were agitated using a sterile needle and the resulting suspension was filtered through three layers of muslin cloth, before adjusting to 2×10^5 spores/ml with the aid of a haemocytometer.

Inoculation: Several dormant terminal buds of clones RRIC 52, 100, 103 and PB 86, grown in the budwood nursery at Dartonfield Estate, were enclosed in transparent polybags and the leaves were allowed to unfurl within the bag to preclude any chance infection. Several healthy 5-day-old leaves were collected, placed in moist chambers and incubated at RT. Using a micro pipette 0.02 ml spore suspension was placed on the adaxial surface of the leaves in an area encircled by a marking pen. As controls, 0.02 ml aliquots of the same suspension were placed on sterilised microscope slides and on slides coated with a thin film of 2% plain agar. These were kept in incubators at RT and removed 3, 6, 9, 24, 48 and 72 h after inoculation. Disks 1.5 cm diameter were punched from the leaves using a cork borer. The leaf disks were placed in McCartney bottles containing alcoholic lactophenol and were prepared as described by Shipton and Brown (1962) and mounted in 50% glycerine for microscope examination. The slides were stained with a drop of cotton blue in lactophenol soon after removal to arrest the growth of the fungus. One hundred spores were counted at random from each of the four replicates to assess germination and germ tube growth was measured in 50 spores. A conidium was considered to be germinated when the length of the germ tube was greater than the breadth of the spore.

Histology: Leaf disks of the clone PB 86 removed at different times were fixed in formalin : acetic acid : alcohol (5:5:90), dehydrated in n-butyl alcohol series and embeded in paraffin (m. pt. 63°C), according to the procedure of Johansen (1940). Sections 8 µm thick were cut with a rotary microtome and stained with safranin and fast green. Photomicrographs were taken using an Olympus light microscope PM-10-A.

RESULTS

After inoculation, on both susceptible and tolerant clones the conidia of *C. gloeosporioides* germinated within 3 h. Typically they produced one germ tube, rarely two, from near one end of the conidium. Initially (after 3 h), more conidia germinated on leaf disks compared to water controls on microscope slides but after 12 h germination was similar on clones of differential susceptibility and on microscope slides. All the conidia on agar-coated slides germinated within 3 h and after 9 h extensive germ tube growth (up to 130 µm) was recorded. By 24 h germ tubes were branching profusely with the production of clusters of secondary conidia, which were not observed on leaf disks incubated for the same period.

Maximum and minimum germ tube development was recorded on leaves of clones PB 86 and RRIC 52, respectively (Fig. 1). However, in the early stages these differences were not marked. The progenies of these two clones viz., RRIC 100 and 103 encouraged an intermediate growth pattern (Fig. 1). Germ tubes grew rapidly on all clones up to 9 h, but then only PB 86 supported good growth.

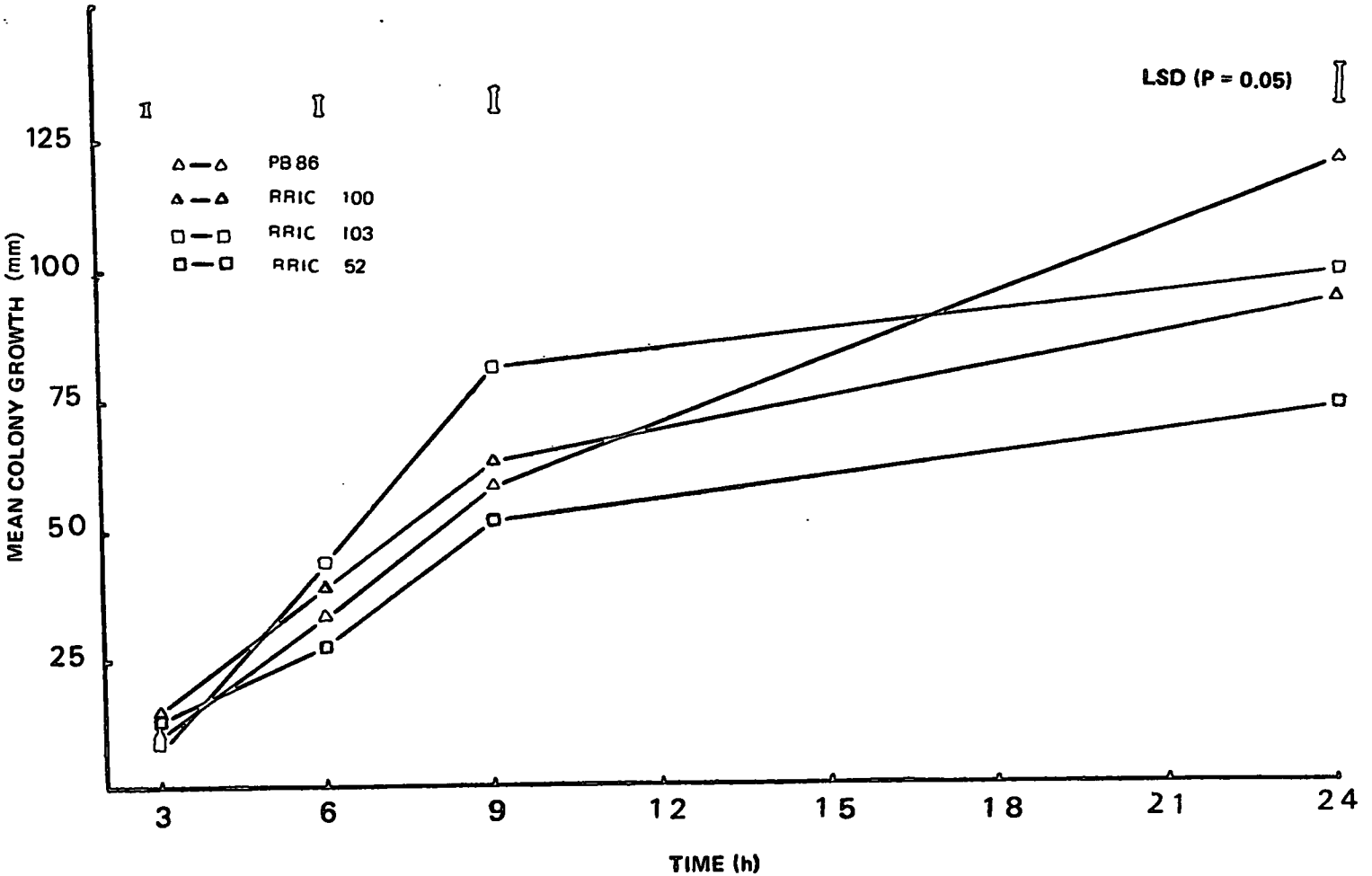


Fig. 1. Colony growth of *C. gloeosporioides* in leaves of different clones

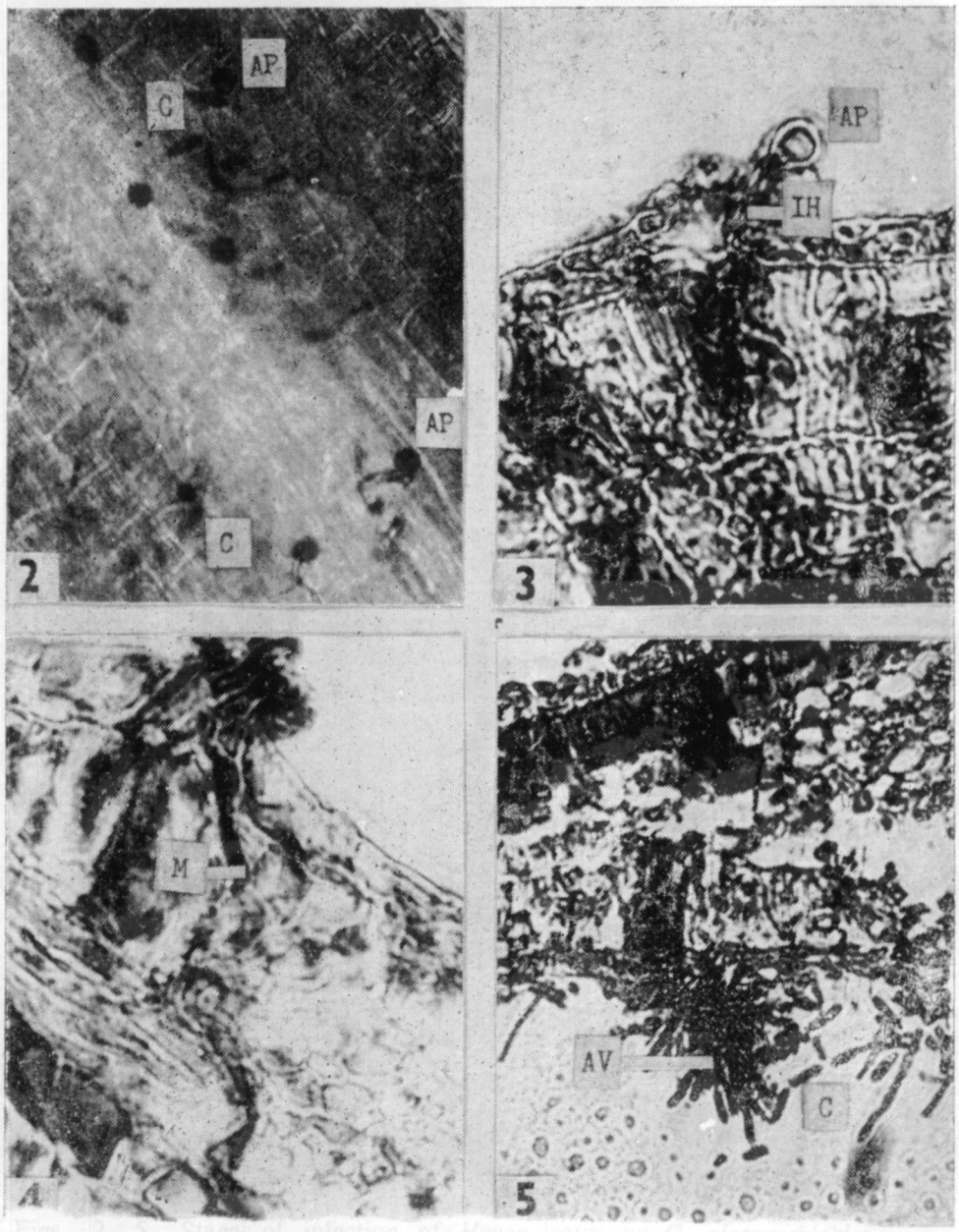
Appressoria were formed abundantly 6 h after inoculation and their numbers increased with prolonged incubation. Occasionally, they were formed earlier on tolerant clones, when they were sessile, with a short germ tube. Appressoria were oval, dark brown with a hard granular covering and measured 7–10 x 4–6 μm. They were distinctly broader than the conidium (Fig. 2) and were usually formed on the anticlinal junctions of adjoining epidermal cells. Significantly more appressoria were formed on RRIC 52 (38.5%) than on PB 86 (23%) after 24 h and there were few on microscope slides and none on agar coated slides.

Before entry into the host tissue through the cuticle and epidermal cell wall, appressoria were separated from the infection hyphae by a septum. Penetration occurred about 9h after inoculation and the infection hyphae entered between the epidermal cells (Fig. 3). They then entered the lumen of the epidermal cells and appeared to enlarge. Often the cells penetrated by the fungus became disorganized. The fungus continued to grow by producing secondary hyphae inter and intra cellularly in the palisade and mesophyll cells. Extensive branching of the mycelium was observed within the mesophyll tissue by 48 h (Fig. 4) and by 72 h the mycelium had reached the lower epidermis. Acervuli developed as sub-epidermal masses of hyphae from which numerous closely packed simple conidiophores arose on the abaxial surface, 72 h after inoculation (Fig. 5). From the apex of each conidiophore one or more conidia were observed. They were hyaline, 1-celled, ovoid to oblong and were embeded together in a mass.

DISCUSSION

Germination of conidia of *C. gloeosporioides* was rapid and showed extensive growth in a short time on agar coated slides followed by the abstriction of secondary conidia from the germ tubes on agar coated slides in 24 h, as recorded by Wimalajeewa (1967). Completion of germination and production of reproductive propagules, in a short period, was possibly due to the presence of an external nutrient source.

Various attempts have been made to explain the physical and chemical factors which induce appressoria formation. While contact stimulus promoted appressorium formation (Netolitzky, 1969) availability of nutrients suppressed it (Mercer *et al*, 1971). The absence of appressoria formation in *C. gloeosporioides* in solutions of sucrose, fructose and glucose, despite extensive mycelial growth could be due to a readily available supply of nutrients (Liyanage & Alwis, 1978). The number of appressoria formed on the leaves of the clone RRIC 52 is nearly double that of PB 86, although the total colony length in the leachate of the former was almost half that of the latter (Liyanage & Alwis, 1978). Appressorial formation and subsequent disease development may thus be explained on the basis that it is dependent on the nutrient status and the balance between stimulatory and inhibitory substances present in the infection drop. This suggests that germintion and development of appressoria are two independent processses, with the latter requiring more specific conditions.Appressoria are frequently formed on the groove at the junction of the anticlinal walls of the epidermal cells of rubber leaves. Similar observations have been made for several species of *Colletotrichum* (Netolitzky, 1969 ; Mercer *et al*, 1971 ; Swinburne, 1976). The appressoria of *C. gloeosporioides* were firmly attached to the host by a mucilagenous secretion as shown for other species of *Colletotrichum* (Marks



Figs. 2 – 5. Stages of infection of *Hevea* leaves by *C. gloeosporioides*.

(2) Germinating conidium (C) with distinct appressorium (AP)
(3) Infection hypha (IH) entering the host cell
(4) Ramification of mycelium (M) within the leaf tissue
(5) Acervuli (AV) formation on the lower leaf surface

et al, 1965). The penetration peg of the appressorium is formed at the point where it comes into contact with the host tissue. It was not possible to determine whether penetration was mechanically or chemically controlled.

Acervuli developed within 72 h after inoculation. This suggests that within 3 days a fresh crop of conidia is produced, thereby increasing the quantity of inoculum, to cause infection of immature leaves.

A great diversity of chemical substances are known to be leached out from plant tissue (Tukey, 1970). Leaves of certain plants may exude fungistatic substances which cause inhibition of spore germination or restrict germ tube growth (Topps & Wain, 1957). The restricted germ tube growth in leachate of clone RRIC 100 as observed by Liyanage & Alwis (1978) may be due to secretion of fungistatic substances like phenols or it may be due to insufficient nutrients that favour mycelial growth. No attempt was made to identify and characterize the substances present in leaf exudates of these clones.

Tan & Low in 1975 reported that more phytoalexins are produced in resistant than in susceptible clones of rubber. It is possible that the different degree of resistance in clones RRIC 52, 100 and 103 may be due to accumulation of phytoalexin like products or substances released from preformed conjugates, after the invasion of host tissues. Cuticle thickness of clones resistant and susceptible to *C. gloeosporioides*, did not show any correlation (Wastie, 1972). Therefore, it appears that resistance to CLD is a complex phenomenon, not dependent on a single factor, but a number of interacting factors, which need further invsetigation.

ACKNOWLEDGEMENTS

I thank the Director, Dr O. S. Peries and Dr David Royle, Long Ashton Research Station UK, for their useful comments on the original manuscript.

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