SUSCEPTIBILITY OF UPPER AND LOWER LEAF SURFACES, AND EFFECT OF WOUNDING OF *HEVEA BRASILIENSIS* (RUBBER) LEAF TO *COLLETOTRICHUM* ISOLATES FROM FOREST TREE

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Species of *Colletotrichum* are amongst the most successful plant pathogenic fungi, attacking an extremely wide range of plants especially in the warm humid tropics. It is known to cause die-back, leaf spots, seedling blight, and leaf blight of several host including tropical acacias (Mordue 1971) and rubber (Wastie 1972). The mechanisms by which *Colletotrichum* species penetrate plant surfaces have been debated for many years. Several modes of penetration are possible—through natural openings, e.g. stomata, through wounds and through direct penetration of the cuticular barrier (Bailey *et al.* 1992). The most common means of penetration is by direct penetration of plant cuticles. Infection through wounds is not common. However, for some diseases, e.g. crown and finger stalk rot of banana, infection through wounds is essential (Krantz *et al.* 1978, Agrios 1988), although in these cases, wounds do not always facilitate infection.

The aim of this study was to determine the susceptibility of upper and lower leaf surfaces and effect of wounding on lesion production in *Hevea brasiliensis* (rubber) leaf by *Colletotrichum* isolates.

In this study, 13 *Colletotrichum* isolates collected from forest trees and rubber were used. Details of these isolates are described in Table 1. Inoculation was done using young, fully expanded leaves of *H. brasiliensis*. The detached leaves were placed flat on a square propylene sheet inside a transparent plastic box (24 × 24 × 2 cm) lined with moist tissue paper. The leaves were carefully handled to avoid damaging their surfaces. Wounding was done prior to inoculation by pricking the upper surface of leaf with a sterile hypodermic needle. Five inoculation sites were pricked on each side of the mid-vein of leaf. Using a microdispenser, the sites were inoculated with 5 to 7 µl drops of conidia suspension (5×10^5 conidia ml^{-1}). Inoculum was also placed on the intact upper and lower surfaces. Droplets of deionised distilled water were used for the control treatments.

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Table 1 The isolate numbers, hosts and symptoms of the 13 *Colletotrichum* isolates used in this study

Isolate no.	Original host	Disease symptom	Collection site
630	*Acacia mangium*	Leaf spots and lesion	FRIM, Malaysia
634	*Hevea brasiliensis*	Leaf spots	Dengkil, Malaysia
635	*Chrysalidocarpus lutescens*	Leaf spots	FRIM, Malaysia
640	*Schizostachym branchycladium*	Leaf spots	FRIM, Malaysia
645	*Magnolia malayana*	Leaf lesions	FRIM, Malaysia
657	*Calamus manan* A	Leaf spots and lesions	FRIM, Malaysia
659	*Calamus manan* B	Leaf spots and lesions	FRIM, Malaysia
660	*Pterocarpus indicus* A	Leaf spots and lesions	FRIM, Malaysia
662	*P. indicus* B	Leaf spots and lesions	FRIM, Malaysia
664	*P. indicus* C	Leaf spots and lesions	FRIM, Malaysia
665	*P. indicus* D	Leaf spots and lesions	FRIM, Malaysia
674	*Schoutenia accrescens*	Leaf spots	FRIM, Malaysia
689	*Gliricida sepium*	Leaf spots	Gualan, Guatemala

The experiments were conducted to compare the time taken for the formation of water-soaked lesions by each isolate. Three leaves were used for each isolate. After inoculation, the transparent plastic boxes containing the inoculated specimens were incubated in a controlled-environment cabinet at 25 °C, 88% relative humidity and 16-hour photoperiod for symptoms to develop. Observations were recorded on alternate days for 12 days and percentages of inoculation sites developing water-soaked lesions were recorded. The results of these experiments are summarised in Figures 1–13.

All isolates produced water-soaked lesions on leaves of *H. brasiliensis.* With the exception of isolate 659 (Figure 7), inoculation of wounded upper surface produced lesions much earlier compared with intact lower and upper surfaces. However, for isolates 665 and 689 (Figures 11 and 13 respectively), there was no difference in terms of time taken for 100% water-soaked lesions to occur between inoculation of wounded and inoculation of the lower surface.

Isolate 665 and 689 were the most pathogenic isolate, causing 100% water-soaked lesions within two days of inoculation of intact lower and wounded upper leaf surfaces. Even on intact upper surface this isolate was highly aggressive, attaining 30% water-soaked lesions within two days.

Intact lower leaf surface inoculated with isolate 659 produced water-soaked symptoms earlier compared with wounded upper surfaces. One possible reason for this is that a high percentage of penetration by this isolate was observed to occur through stomata (Figure 14). Observations using SEM revealed that most of the stomata were present on the lower surface of the leaf, increasing penetration opportunities. Penetration through stomata is actually rare in species of *Colletotrichum* but similar observation had been reported by Senechal *et al.* (1987). There were no stomata on the upper surface except for those situated on the veins.

Generally the order of presence of water-soaked lesions are wounded upper surface > intact lower surfaces > intact upper surface. This suggests that wounding greatly increase host susceptibility regardless of the isolate.

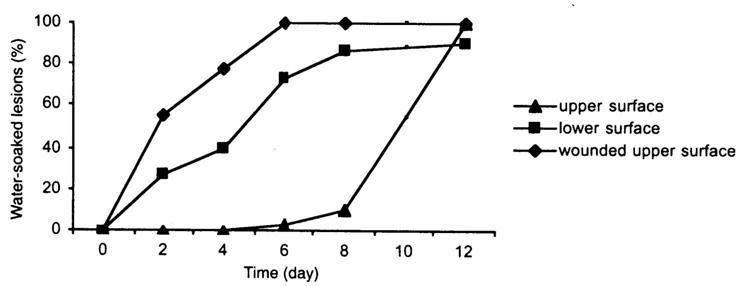

Figure 1 Symptom development on different leaf surface of *Hevea brasiliensis* inoculated with isolate 630

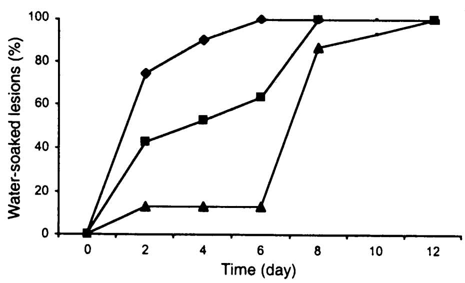

Figure 2 Symptom development on different leaf surface of *H. brasiliensis* inoculated with isolate 634

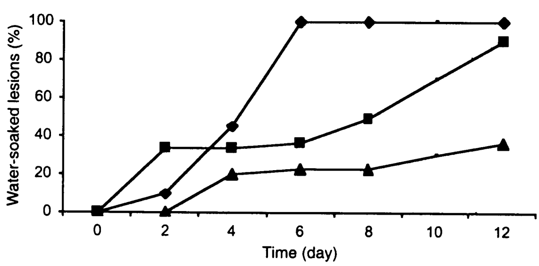

Figure 3 Symptom development on different leaf surface of *H. brasiliensis* inoculated with isolate 635

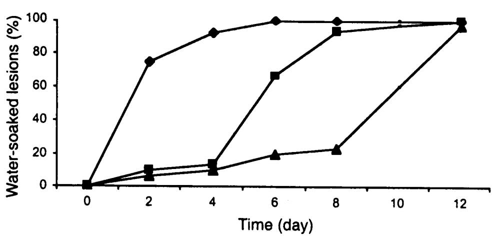

Figure 4 Symptom development on different leaf surface of *H. brasiliensis* inoculated with isolate 640

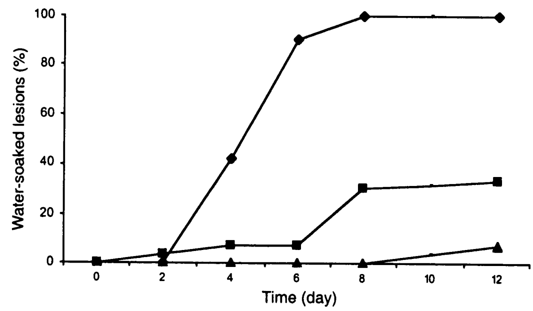

Figure 5 Symptom development on different leaf surface of *H. brasiliensis* inoculated with isolate 645

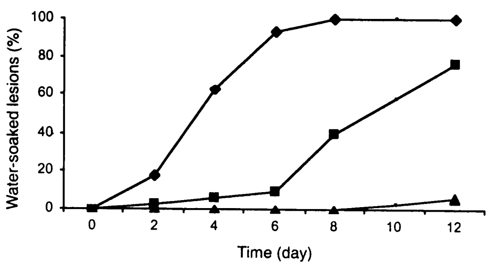

Figure 6 Symptom development on different leaf surface of *H. brasiliensis* inoculated with isolate 657

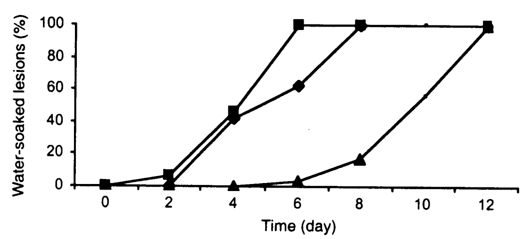

Figure 7 Symptom development on different leaf surface of *H. brasiliensis* inoculated with isolate 659

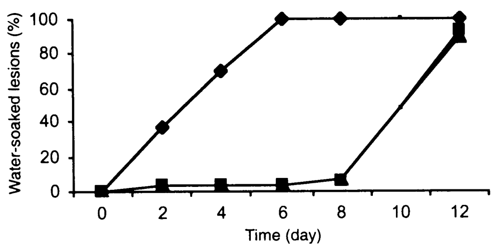

Figure 8 Symptom development on different leaf surface of *H. brasiliensis* inoculated with isolate 660

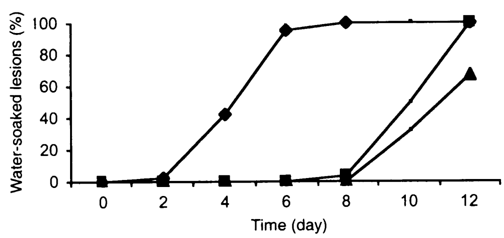

Figure 9 Symptom development on different leaf surface of *H. brasiliensis* inoculated with isolate 662

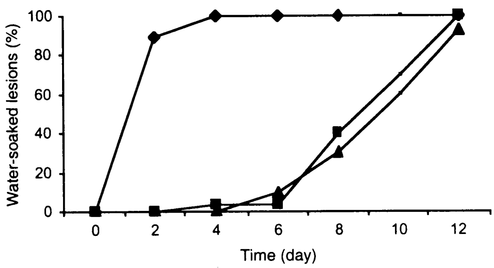

Figure 10 Symptom development on different leaf surface of *H. brasiliensis* inoculated with isolate 664

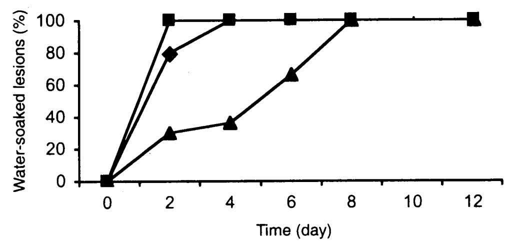

Figure 11 Symptom development on different leaf surface of *H. brasiliensis* inoculated with isolate 665

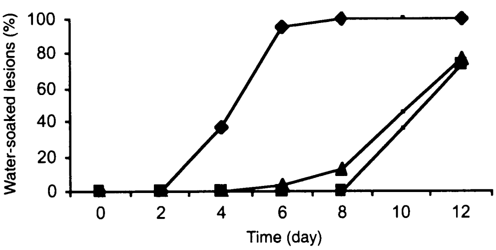

Figure 12 Symptom development on different leaf surface of *H. brasiliensis* inoculated with isolate 674

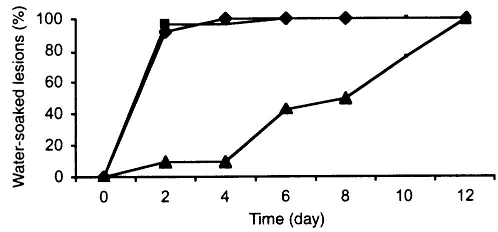

Figure 13 Symptom development on different leaf surface of *H. brasiliensis* inoculated with isolate 689

It is still not clear how wounding increase susceptibility as infection through wounding is not common in species of *Colletotrichum*, although it may be related to natural infection process. One possibility is easier access to dead cell wall which provide an easy entrance for the pathogen. Bailey *et al.* (1992) reported that wounding did not induce infection by *C. lindemuthianum* on susceptible bean hypocotyls. In contrast, our results conform to the findings of Zakaria (1990) and Johnson and Miliczky (1993).

Figure 14 Penetration through stomata by isolate 659: conidia (C), germtube (GT) and stomata (S)

References

AGRIOS, G. N. 1988. *Plant Pathology*. 3rd edition. Academic Press Inc., New York. 803 pp.

BAILEY, J. A., O'CONNELL. R. J., PRING, R. J. & NASH, C. 1992. Infection strategies of *Colletotrichum* species. Pp. 88–120 in Bailey, J. A. & Jeger, M. J. (Eds.) *Colletotrichum: Biology, Pathology and Control*. CAB International, Wallingford.

JOHNSON, A. & MILICZKY, E. R. 1993. Effects of wounding and wetting duration on infection of potato foliage by *Colletotrichum coccodes*. *Plant Disease* 77: 13–17.

KRANTZ, J., SCHMUTTERER, H. & KOCH, W. 1978. *Diseases, Pests and Weeds in Tropical Crops*. Wiley and Sons, Chichester.

MORDUE, J. M. 1971. *CMI Descriptions of Pathogenic Fungi and Bacteria*. Phamphlet Nos. 315, 316, 317.

SENECHAL, Y., SANIER, C., GOHET, E. & D'AUZAC, J. 1987. Pp. 537–542 in *Different Modes de Penetration* of Colletotrichum gloeosporioides *dans les feuilles* d'Hevea brasiliensis. Comptes Rendues de l'Academie Science, Paris 305, Ser. 3.

WASTIE, R. L. 1972. Secondary leaf fall of *Hevea brasiliensis*: meteorological and other factors affecting infection by *Colletotrichum gloeosporioides*. *Annals of Applied Biology* 72: 283–293.

ZAKARIA, M. 1990. Comparative studies of three *Colletotrichum capsici* isolates with reference to their morphology and pathogenicity. M.Sc. thesis, University of Bristol, Bristol. 41 pp.