

MYCOFLORA ASSOCIATED WITH *HEVEA* PHYLLOPLANE

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SUMMARY

Eleven genera of fungi namely Aspergillus, Botryodiplodea, Cladosporium, Colletotrichum, Curvularia, Fusarium, Mucor, Penicillium, Phomopsis Pestalotiopsis, Trichoderma were isolated from healthy leaves. Surface sterilized lesions from infected leaves frequently yielded Botryodiplodea, Cladosporium, Colletotrichum, Curvularia, Fusarium, Periconia, Pestalotiopsis and Phomopsis while genera like Trichoderma, Aspergillus, Mucor, Penicillium, Alternaria and Beltrania were also isolated occasionally in addition to the primary cause of the respective lesion.

Key words: *Hevea brasiliensis*, disease lesions, mycoflora.

The surface of healthy leaves of plants are colonized by a wide range of micro organisms. Most of these organisms are believed to be non-parasitic but some are common pathogens (Kangsen & Kapooria, 1979). Today leaf inhabiting microflora of most of the tropical and temperate plants are well documented and studies covered various aspects (Hudson, 1962; Kendrick & Burges, 1962; Cadwall, 1963).

The most widely covered aspect is the successional pattern of the micro-biota (Hudson, 1962; Kendrick & Burges, 1962; Cadwall, 1963; Kangsen & Kapooria, 1979 and Gunasekara, 1994) and it is believed that the phylloplane flora has a definite successional pattern (Hogg & Hudson, 1966; Hudson, 1962; Kendrick & Burges, 1962 and Kangsen & Kapooria, 1979). A recent interesting development on saprophytic flora on leaf surface is their interactions with pathogens and potential use of them as biological control agents (Kapooria & Sinha, 1969; Fokkema & Vander Meulen, 1976; Bashi & Fokkema, 1977 and Blakeman & Brodie, 1977). Further, instances where production of plant growth promoting auxins by yeast like fungi (Buckley & Pugh, 1971) and nitrogen fixation on the leaf surface by free living nitrogen fixing bacteria (Jones, 1970) have also been reported.

Despite many investigations on other plant species, little is known about the phylloplane mycoflora and their interactions in *Hevea*. Kangsen & Kapooria in 1979

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have studied the succession of rubber phylloplane microbiota in Zambia and several incomplete studies have been made on the phylloplane flora of rubber in Sri Lanka (Liyanage & Dharmaratne, 1983; Jayasinghe *et al.*, 1996).

This paper documents the phylloplane mycoflora of both healthy and infected leaves of *Hevea* rubber in Sri Lanka.

Healthy rubber leaves of different maturity were collected from all rubber growing areas and cut these leaves into small pieces. All isolations were made on potato dextrose agar (PDA) plates as it is a general purpose medium. Five leaf pieces around 2mm diameter were introduced into each plate and incubated under normal light and dark regimes at RT ($28\pm 2^{\circ}\text{C}$). More than 500 isolates were obtained over a period of six months. Pure cultures were obtained from the colonies appeared up to six days.

Isolations were made from diseased tissues caused by common pathogens namely *Colletotrichum*, *Oidium* and *Corynespora* after subjecting to surface sterilization (3 min in 70% ethanol). The mycoflora resulted in addition to the primary cause were also recorded. Finally, the vegetative and the reproductive morphology of the resulted cultures were studied using CMI descriptions published by CABI Bioscience, U.K.

Eleven genera of fungi were isolated consistently from the healthy rubber leaves. The most abundant were species of *Aspergillus*, *Botryodiplodea*, *Cladosporium*, *Colletotrichum*, *Curvularia* and *Fusarium*. Fungal genera such as *Pestalotiopsis*, *Penicillium*, *Phomopsis*, *Mucor* and *Trichoderma* were not common as the other genera recorded. Most of these fungi also appeared when isolations were made from the surface sterilized lesions caused by known pathogens. However, genera like *Trichoderma*, *Aspergillus*, *Mucor* and *Penicillium* were noticed very rarely on the diseased lesions under the surface sterilized conditions. The only additional genera isolated from diseased tissue were *Alternaria* and *Beltrania*. They also appeared occasionally (Table 1). The colony characteristics and conidial morphology of the isolated fungi are given in tabular form (Table 2) and microphotographs of the important genera are also provided (Fig. 1 & 2).

It should be noted that some of the genera isolated from healthy leaf tissue namely *Colletotrichum* spp. (Petch, 1905; Jayasinghe *et al.*, 1997), *Botryodiplodea* (Petch, 1911), *Fusarium* (Weir, 1926) and *Pestalotiopsis* (Petch, 1911) are also considered on pathogens of *Hevea* rubber.

Table 1. *Fungal genera isolated from healthy tissue and surface sterilized diseased lesions (in addition to the primary cause of the lesion)*

Genera isolated	Healthy tissue	Diseased lesions
<i>Alternaria</i>	-	+
<i>Aspergillus</i>	+	+
<i>Beltrania</i>	-	+
<i>Botryodiplodea</i>	+	++
<i>Cladosporium</i>	+	++
<i>Colletotrichum</i>	+	++
<i>Curvularia</i>	+	++
<i>Fusarium</i>	+	++
<i>Mucor</i>	+	+
<i>Penicillium</i>	+	+
<i>Periconia</i>	-	++
<i>Pestalotiopsis</i>	+	++
<i>Phomopsis</i>	+	++
<i>Trichoderma</i>	+	+

+ occasionally observed
 ++ frequently observed
 - absent

Table 2. *Colony characteristics and the conidial morphology of common mycoflora associated with Hevea phylloplane*

Fungal species	Colony morphology	Conidial characters
<i>Aspergillus</i>	Rapidly growing colonies are in different shades of green in colour.	Conidiophores are hyaline with globular vesicles and conidia are (1.5 – 2.5) μm in size.
<i>Alternaria</i>	White to greenish brown colonies with greenish patches.	Conidial beak was characteristic, elongate conidia with transverse and longitudinal septa (40-80 X 10-30) μm .
<i>Beltrania</i>	White to grey colonies with an uneven margin.	Abundantly sporulated. Apical appendage observed. Hyaline, single septate and (25-35 X 8-10) μm in size.

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Fungal species	Colony morphology	Conidial characters
<i>Cladosporium</i>	Dark green coloured colony	Produce abundant conidia, fusiform, cylindrical, ovate or ellipsoidal, showing a wide range of shapes (2.5-10 X 2.5-5) μm in size.
<i>Colletotrichum</i> spp.	Colonies white to dark grey with aerial mycelium eg. <i>C. gloeosporioides</i> Colonies white to orange in colour eg. <i>C. acutatum</i>	hyaline, cylindrical spores with rounded ends (10-25 X 3-6) μm in size. hyaline, fusiform in shape and (7-12 X 5-7) μm in size.
<i>Curvularia</i>	Colony greyish black with a slight shade of green	Conidia slightly curved, pigmented, three septate and (17-25 X 7-10) μm in size.
<i>Fusarium</i>	White colonies later become slight pinkish in colour with moderate aerial mycelium	Plenty of macroconidia, hyaline, mostly 3 septate and (12-25 X 3) μm in size. Micro conidia aseptate, slightly elongate and (8-12 X 5-8) μm in size.
<i>Mucor</i>	Fast growing colonies are grey to black in colour	Globose sporangia are produced abundantly, spores are globular and (4-7) μm in size.
<i>Penicillium</i>	Colonies are in different shades of green with a white margin.	Plenty of conidial heads seen, conidiophores branched, phialides prominent and globular conidia (2.5-3) μm in size.
<i>Periconia</i>	Slow growing colonies are greenish grey in colour with plenty of aerial mycelium.	Conidiophores erect, pigmented, (4.5-10) μm globular spores are arranged on it.
<i>Pestalotiopsis</i>	Colony is white with aerial mycelium and an uneven advancing margin. Diurnal zonation is characteristic and black spore masses are seen here and there.	Spores are strait, five celled, (20-25 X 7-9) μm in size. Basal cells are hyaline and three apical appendages are observed.
<i>Phomopsis</i>	Colony initially white later turning into greyish in colour. Black coloured conidial masses are scattered.	Two types of conidia viz. fusiform, hyaline (4-5 X 2-3) μm and hyaline filiform, curved, aseptate (15-20) μm in size.
<i>Trichoderma</i>	Initially colony is white and fast growing. Later, plenty of greenish conidial masses seen.	Hyaline, single celled, globular conidia (2.5-4) μm in size.

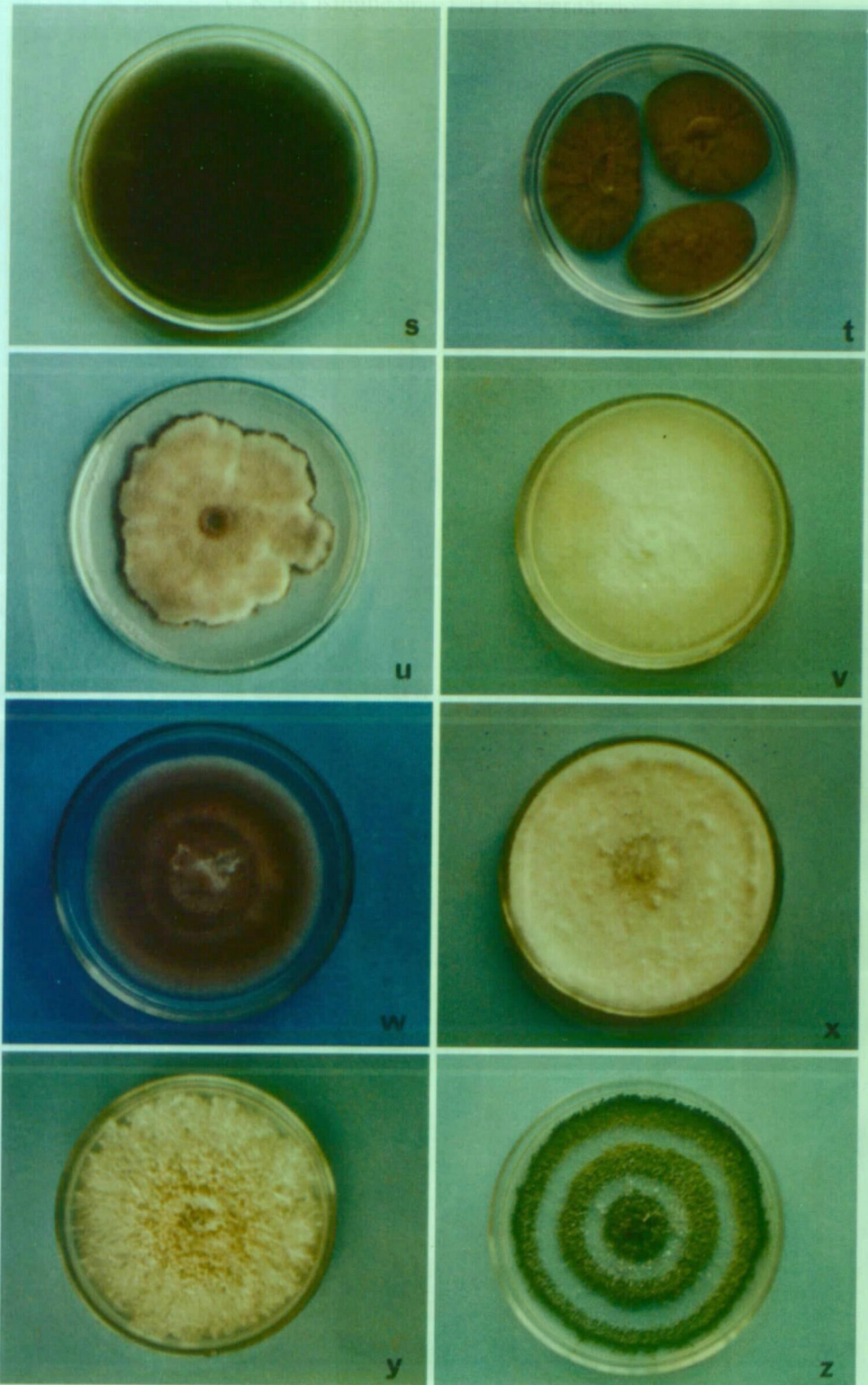
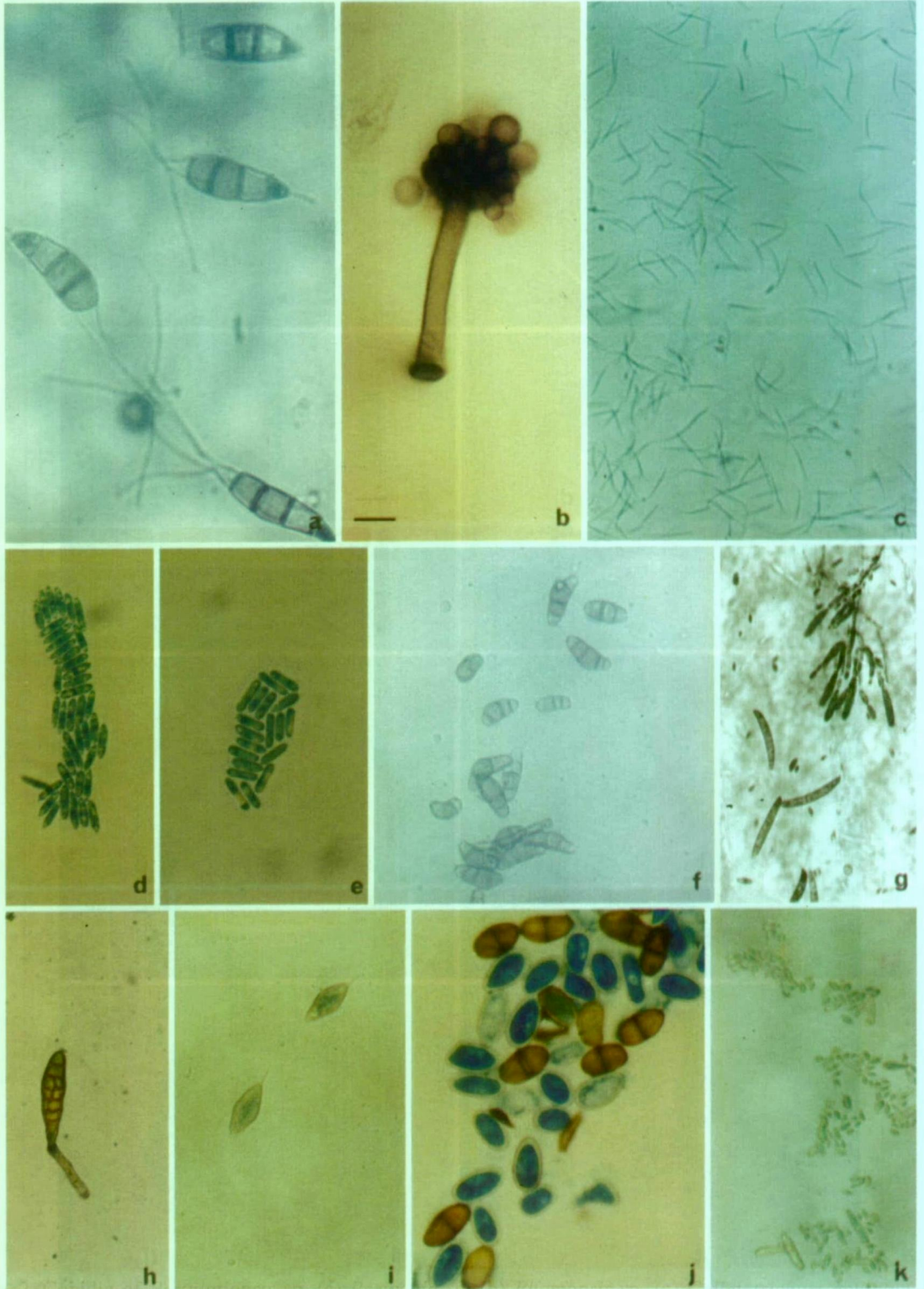


Fig. 1. Colonies of *Botryodiplodia* sp. (s), *Cladosporium* sp. (t), *Curvularia* sp. (u), *Fusarium* sp. (v), *Periconia* sp. (w), *Pestalotiopsis* sp. (x), *Phomopsis* sp. (y) and *Trichoderma* sp. (z)

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Fig. 2. Conidia of *Pestalotiopsis* sp. (a) *Periconia* sp. (b) *Phomopsis* sp. (c) *Colletotrichum acutatum* (d) *Colletotrichum gloeosporioides* (e) *Curvularia* sp. (f) *Fusarium* sp. (g). *Alternaria* sp. (h) *Beltrania* sp. (i) *Botryodiplodia* sp. (j) and *Cladosporium* sp. (k). Scale bar in (b) represents 20 μ m in (c) (d) (e) (f) (g) (h) (i) (j) & (k) and 8 μ m in (a) & (b).

REFERENCES

- Bashi, E and Fokkema, N J** (1977). Environmental factors limiting the growth of *Sporobolomyces roscus*, an antagonist of *Cochliobolus sativas*, on wheat leaves. *Transactions of the British Mycological Society* **66**, 17-25.
- Blakeman, J P and Brodie, I D S** (1977). Competition for nutrients between epiphytic micro-organisms and germination of spores of plant pathogens on beet root leaves. *Physiological Plant Pathology* **10**, 29-42.
- Buckley, N G and Pugh, G H F** (1971). Cultural studies of leaf saprophytes. In: *Ecology of leaf surface micro-organisms*. pp. 77-100 (Eds. T F Preece and Dickinson).
- Fokkema, N J and Vander Meulen** (1976). Antagonism of yeast like phyllosphere fungi against *Septoria hodorum* on wheat leaves. *Netherland Journal of Plant Pathology* **82**, 13-16.
- Gunasekara, T S** (1994) Isolation and enumeration of microbial inhabitants of tea phylloplane. *Sri Lanka Journal of Tea Science* **63**, 19-27.
- Hogg, B M and Hudson, H F** (1966). Micro fungi on leaves of *Fagus sylvaticui*. The micro fungal succession. *Transactions of the British Mycological Society* **49**, 185-192.
- Hudson, H J** (1962). Succession of micro fungi on ageing leaves of *Saccharum officinarum*. *Transactions of the British Mycological Society* **45**, 395-423.
- Jayasinghe, C K, Fernando, T H P S and Pradeepika, O A N** (1996). Studies on the phylloplane microflora of *Hevea brasiliensis*. *Annual Review of the Rubber Research Institute of Sri Lanka* **52**.
- Jayasinghe, C K, Fernando, T H P S and Priyanka, U M S** (1997). *Colletotrichum acutatum* is the main cause of *Colletotrichum* leaf disease of rubber in Sri Lanka. *Mycopathologia* **137**, 53-56.
- Kangsen, I N and Kapooria, R G** (1979). Studies on the succession of phylloplane microflora of developing leaves of *Hevea* rubber. *Tropical Ecology* **20**, 41-48.
- Kapooria, R G and Sinha, S** (1969). Phylloplane mycoflora of pearl millet and its influence on the development of *Puccinia penniseti*. *Transactions of British Mycological Society* **53**, 153-155.
- Kendrick, W B and Burges, A** (1962). Biological aspects of the decay of *Pinus sylvestris* leaf litter. *Nova Hedwigia* **4**, 314-344.
- Liyanage, A de S and Dharmarathna, A** (1983). Microflora on rubber leaves. *Annual Review of the Rubber Research Institute of Sri Lanka*. **56**.
- Petch, T** (1905). Description of new Ceylon Fungi. *Annual Report of Botanical Gardens, Peradeniya*. **3**, 1-10.
- Petch, T** (1911). The physiology and diseases of *Hevea brasiliensis*, the premier plantation rubber tree. London, 268pp.

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