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Mycorrhizal status of epiphytes in Malaysian oil palm plantations

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Abstract. Epiphytic plants were collected from four oil palm plantations in Peninsular Malaysia and their mycorrhizal status determined. Conspecific plants with a terrestrial habit (16 species) and rhizosphere soils were also examined for mycorrhizal colonization and glomalean fungi, respectively. Twelve species of glomalean fungi were recovered from the four oil palm plantation soils. Of the 29 epiphytic species in 16 families belonging to the bryophytes, pteridophytes and angiosperms, only four species of angiosperms that were facultative epiphytes and a hemiepiphyte growing within 0.4 m of ground level had vesicular-arbuscular mycorrhizal (VAM) fungi. Bioassays of organic debris from oil palm trunks did not produce vesicular-arbuscular mycorrhizas on maize. Six epiphytic species grown in the greenhouse in pots containing oil palm rhizosphere soils rooted and had VAM fungi and thus may be facultative epiphytes. Five other epiphyte species failed to grow in pots and are probably obligate epiphytes. Seven epiphyte species that established themselves in pots failed to form vesicular-arbuscular mycorrhizas.

Key words: Epiphytes – Vesicular-arbuscular mycorrhizas – Pteridophytes – Malaysian plantations – Glomales

Introduction

Malaysia is an agricultural country with three major commercial crops: rubber (*Hevea brasiliensis* Muell. Arg.), oil palm (*Elaeis guineensis* Jacq.) and cocoa (*Theobroma cacao* L.), together estimated to occupy about 3 million hectares. Of these, oil palm, the most profitable tropical crop, occupies approximately 1845700 hectares (Karim 1990). A survey of the soils of oil palm plantations revealed species of all six genera of the Glomales (Nadarajah 1980 and unpublished work). Although the roots of oil palm have vesicular-arbuscular mycorrhizal (VAM) fungi (Nadarajah 1980; Bial and Gianinazzi-Pearson 1989), little is known of the mycorrhizal associations of epiphytes occurring in association with oil palms.

The trunk of oil palm is an erect, solitary column, the surface of which is protected by persistent leaf bases. A number of plants such as lichens, algae, mosses, ferns and angiosperms grow as epiphytes and climbers in these leaf bases and on the surface of the trunk. The fern flora (both epiphytic and terrestrial) in oil palm estates was recorded by Piggott (1980).

Research on the mycorrhizal relationships of epiphytes is relatively recent. In this present study, a survey of the mycorrhizal status of epiphytic bryophytes, ferns and angiosperms occurring in oil palm plantations in West Malaysia is reported.

Materials and methods

This study was conducted at four sites in three locations near Kuala Lumpur in Peninsular Malaysia. Their soil classification, pH measured in water (10 g: 25 ml water) and available Bray 2 phosphorus are presented in Table 1. The soils were acidic and the available P content ranged from low to medium. These areas have air temperatures of approximately 25 to 39° C and the annual rainfall is 2680 mm. Except for site P4, three of the four sites are well-managed commercial plantations.

Samples of epiphytes were collected from all four oil palm plantations, but most species were collected from site P4, University of Malaya, because this plantation is not commercially maintained. In well-managed plantations, regular pruning of fronds and weeding occur and the bases and trunks of oil palms are regularly cleared of weeds, cover crops, creepers and ferns, although some species of epiphytes and climbers still establish. Exposed trunks of oil palms that were not well maintained, especially at site P4, were covered by epiphytes and climbers, although there were only a few epiphytes on the upper region of the trunk covered by old fronds. A few species of bryophytes (*Barbula indica* and *Bryum nitens*) and angiosperms (*Axonupus compressus*, *Borreria alata*, *Peperomia pellucida* and *Phyllanthus niruri*) which are typically strictly terrestrial were found growing on the trunks of oil palms within 0.4 m of the ground. *Ficus* sp. (probably *F. benjamina*) and *Asplenium nidus* were found occasionally at a height of about 1.6 m above the ground.

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Table 1. Location of study sites and some properties of soils in oil palm plantations

Location	Site number	Soil classification: Order	Soil classification: Series	pH	Bray 2P (ppm)
Prang Besar Estate, Kajang	P1	Oxisol	Munchong	4.5	15
	P2	Oxisol	Prang	4.6	8
Malaysian Agricultural Development and Research Institute, Serdang	P3	Ultisol	Serdang	3.9	11
University of Malaya, Kuala Lumpur	P4	Ultisol	Serdang	4.3	5

Based on their growth habitat on porophytes (supporting trees), Lesica and Antibus (1990) recognized three types of epiphytes: trunk, bare-limb and canopy. In this study, all the epiphytic species collected are trunk epiphytes (Table 2). Canopy epiphytes other than crustose lichens were not found at any of the sites. Because oil palms do not form branches, bare-limb epiphytes cannot occur.

Conspecific epiphytes were collected from the same tree or nearby trees. Root samples from at least 10 individuals of each species of bryophyte and pteridophyte, and from four plants of each angiosperm species were carefully washed free of organic debris and fixed in formalin/acetic acid. Roots of conspecifics growing as terrestrial plants were also collected when present. Organic debris around the epiphytes, especially from the retaining leaf bases in the middle region of the oil palm trunk, and rhizosphere samples around terrestrial conspecifics of epiphytes were collected. Glomalean fungus spores were separated from soil by wet-sieving and decanting (Gerdemann and Nicolson 1963).

Preserved roots were rinsed in tap water and cleared in 10% KOH at about 90° C for 1 to 2 h. Dark roots were bleached in hydrogen peroxide for 20 min. All roots were acidified with HCl and stained with trypan blue (Phillips and Hayman 1970). Individual roots were examined at ×40–100 magnification for the presence of arbuscules, coils, vesicles, internal hyphae and external hyphae of VAM fungi. Mycorrhizal colonization was estimated by the grid-line intersect method (Giovannetti and Mosse 1980).

A bioassay for mycorrhizal fungi present in organic debris from oil palm trunks was attempted using maize (*Zea mays* L.) as a host. Organic accumulations from the leaf bases of oil palm were collected where either of two bryophytes (*Calymperes erosum* and *Leucophanes octoblepharioides*) or the pteridophytes listed under "epiphytic" in Table 2 were growing. The organic material was mixed with sterilized sand and six replicate pots were prepared. Six pots containing only sterilized sand were used as a control. The maize plants were grown in a greenhouse for 12 weeks. After harvest, roots were examined for root colonization.

Some of the field-collected epiphytes comprising two species of bryophytes and 16 species of ferns (listed under "greenhouse" in Table 2) were free of mycorrhizas. These mycorrhiza-free individuals were transplanted to pots containing terrestrial rhizosphere soils from oil palm plantations. These plants were grown for 3 months in the greenhouse to test for mycorrhizal colonization.

Results

Species of epiphytes and their terrestrial conspecifics are listed in Table 2 with their collection sites and mycorrhizal status. A total of 29 species belonging to 16 families was examined for the presence of mycorrhizal fungi: four species of bryophytes, 19 species of pteridophytes, and six species of angiosperms. All epiphytic species of bryophytes and pteridophytes lacked mycorrhizas. Of the six species of angiosperms, only four species in four families growing within 0.4 m from the ground had VAM fungi, with levels of colonization below 30%. Vesicles, coils, internal hyphae and external hyphae were present in most species showing mycorrhizal root colonization, however, arbuscules were not observed. We only observed coarse types of VAM fungi on epiphyte species, although we did find other nonmycorrhizal fungi and their spores associated with epiphyte roots.

Except for the bryophytes, most terrestrial conspecifics of epiphytes had VAM fungi. Vesicles, coils, internal hyphae and external hyphae of VAM fungi were present in most species of pteridophytes and angiosperms, but arbuscules were few in angiosperms such as *Axonopus compressus*, *Phyllanthus niruri* and *Peperomia pellucida*, and were not observed in pteridophytes.

Infection of maize plants with organic debris collected from the vicinities of epiphytes failed to produce mycorrhizas. The organic debris was not sieved for spores of glomalean fungi.

Spores of 12 species of Glomales belonging to *Glomus* (*G. clarum*, *G. clavisporum*, *G. fasciculatum*, *G. geosporum*, *G. macrocarpum*, *G. microcarpum* and an unidentified *Glomus* sp.), *Sclerocystis* (*S. coremioides*), *Acaulospora* (*A. laevis* and *A. scrobiculata*) and *Gigaspora* (*G. decipiens* and *G. margarita*) and auxiliary cells belonging to the latter genus were isolated from sievings of terrestrial rhizosphere soils. The "fine endophyte", *Glomus tenue* (Greenall) Hall, was not encountered in any of the oil palm plantations soils. The average spore number per 100 g soil was 114, 105, 109 and 57 for sites P1, P2, P3 and P4, respectively.

The mycorrhizal status of some transplants of mycorrhiza-free epiphytes grown in oil palm plantation terrestrial soils in the greenhouse is presented in Table 2. Five species of ferns failed to grow in soil. The rhizoids of two species of bryophytes and five species of pteridophytes which were grown in the greenhouse had no mycorrhizal colonization. Six other species of ferns had VAM fungi with vesicles and/or coils, but arbuscules were not observed.

Discussion

Bermudes and Benzing (1989) and Lesica and Antibus (1990) examined the fungi associated with epiphytes at three Ecuadorian locations and in two Costa Rican forests, respectively. Their studies described many species of angiosperms, including members of the Ericaceae

Table 2. Mycorrhizal status of epiphytes and conspecific terrestrial plants from oil palm plantations and of some mycorrhiza-free epiphytes grown in the greenhouse

Species	Site number	Growth habit[a]	Mycorrhizal colonization, % with structures (±SD)		
			Epiphytic	Terrestrial	Greenhouse
Bryophyta					
Bryaceae					
Bryum nitens Hook.	P4	e, t	0	0	0
Calymperaceae					
Calymperes erosum C. Mull.	P4	E	0	—[b]	y[d]
Leucobryaceae					
Leucophanes octoblepharioides Brid.	P4	E	0	—	y
Potticeae					
Barbula indica (Hook.) Spreng.	P4	e, t	0	0	0
Pteridophyta					
Adiantaceae					
Adiantum latifolium Lam.	P4	e	0	0	0
Taenitis blechnoides (Willd.) Sw.	P4	e, T	0	20±7 (h, c, v)[c]	25±8 (h, c)
Vittaria elongata Sw.	P4	E	0	—	y
Dennstaedtiaceae					
Asplenium nidus L.	P4	e	0	—	0
Davallia denticulata (Burm.) Mett.	P1–P4	E, t	0	13±6 (h, v)	0
Nephrolepis biserrata (Sw.) Schott.	P1–P4	E, T	0	30±8 (h, c, v)	20±3 (h, c, v)
Pteris ensiformis Burm.	P3, P4	e, t	0	19±8 (h, v)	y
Stenochlaena palustris (Burm.) Bedd.	P1	E, T	0	10±6 (h, v)	41±7 (h, c)
Tectaria maingayi (Bak.) C. Chr.	P1	e, T	0	14±4 (h, c, v)	32±5 (h, c)
Polypodiaceae					
Drymoglossum piloselloides (L.) Pr.	P1–P4	E	0	—	z[e]
Drynaria quercifolia (L.) J. Sm.	P4	e	0	—	y
Drynaria sparsisora (Desv.) Moore	P4	e	0	—	z
Phymatodes scolopendria (Burm.) Ching	P4	e	0	—	z
Plactycerium coronarium (Koenig.) Desv.	R3	e	0	—	z
Polypodium verrucosum (Hook.) Wall	P1, P4	E, t	0	22±5 (h, c, v)	14±5 (h, v)
Pyrrosia angustata (Sw.) Ching	P4	e	0	—	z
Psilotaceae					
Psilotum nudum (L.) Beauv.	P2, P4	e	0	—	0
Schizaeaceae					
Lygodium flexuosum (L.) Sw.	P3, P4	e, T	0	29±8 (h, c, v)	17±8 (h, c)
Selaginellaceae					
Selaginella rivalis Ridl.	P4	e, t	0	—	0
Angiospermae					
Monocotyledoneae					
Araceae					
Scindapsus aureus Engl.	P4	e, t	0	20±4 (h, v)	y
Gramineae					
Axonopus compressus (Sw.) P. Beauw.	P4	e, T	7±2 (h, c)	32±5 (h, a, c, v)	y
Dicotyledoneae					
Euphorbiaceae					
Phyllanthus niruri L.	P4	e, T	10±3 (h, v)	15±5 (h, a, c, v)	y
Moraceae					
Ficus sp.	P4	e	0	—	y
Piperaceae					
Peperomia pellucida (L.) H.B.K.	P4	e, T	24±6 (h, c, v)	35±6 (h, a, c)	y
Rubiaceae					
Borreria alata (Aubl.) DC.	P4	e, t	12±7 (h, c)	17±6 (h, v)	y

[a] For the growth habit of epiphytes on oil palm trunks: E, an epiphyte is common, abundant or of frequent occurrence; e, an epiphyte of less frequent occurrence; T, a terrestrial plant common on the floor of the oil palm plantation, locally abundant or frequent; t, a terrestrial plant of less frequent occurrence

[b] Conspecific terrestrial plant absent

[c] Structures of vesicular-arbuscular mycorrhizas: a, arbuscule; c, hyphal coil; h, hypha; v, vesicles

[d] Plants not grown in pots in the greenhouse

[e] Epiphytes which were mycorrhiza-free transplants failed to establish in pots containing plantation soils

and Orchidaceae which have their own kinds of mycorrhizas, in the epiphytic population. In contrast, the majority of epiphytic species in Malaysian oil palm plantations are ferns. In well-maintained oil palm plantations, most epiphytes are removed from trunks to prevent smothering effects and growth among the inflorescences and fruits. In unmanaged oil palm plantations, however, the rhizoids of mosses and ferns form dense organic mats on trunks, supporting and encouraging the growth of many other species of epiphytes.

The limited occurrence of VAM fungi was reported by Bermudes and Benzing (1989) and Lesica and Antibus (1990). Of the five species of Araceae examined by Lesica and Antibus (1990), only one species had VAM fungi. In the present study, and that of Bermudes and Benzing (1989), epiphytic members of Araceae lacked mycorrhizas. Some species of *Peperomia* (Piperaceae) had VAM fungi as observed also by Benzing and Bermudes (1989) but not by Lesica and Antibus (1990). In this study and that of Lesica and Antibus (1990), only one species of *Ficus* was examined and this lacked mycorrhizas. The genera of ferns examined in this study and that of Lesica and Antibus (1990) were *Asplenium*, *Polypodium* and *Selaginella* and all epiphytic species of these genera lacked mycorrhizas.

Of the 29 epiphytic species examined in the present study, 13 are obligate epiphytes (only growing as epiphytes), 15 are facultative epiphytes (growing either as epiphytes or as terrestrial plants) and *Scindapsus aureus* can be considered as a hemiepiphyte (growing as an epiphyte while also rooting in the ground). Except for four species of angiosperms which were growing near ground level, all other epiphytes lacked mycorrhizas, in agreement with the finding of Lesica and Antibus (1990).

Lesica and Antibus (1990) suggested that the paucity of VAM fungi in epiphytic habitats could be due to the inability of these fungi to disperse to arboreal habitats, or the fact that many epiphyte habitats are too exposed and dry for glomalean fungi to survive. Nevertheless, in addition to epiphytic plants, rats, snakes and other small animals also make their homes in the retained leaf bases of oil palms (Piggot 1980) and might carry spores to these locations. Ants were seen on trunks in the abandoned oil palm plantation at site P4. The VAM fungi colonization of plants within 0.4 m of the ground could be due to dispersal of propagules of mycorrhizal fungi by these organisms, or because of rain splash of soil and spores. Organic debris from trunks was not sieved, however, so the occurrence of spores of glomalean fungi is not known. Oil palm trunks with their persistent leaf bases and organic mats are humid, especially during rainy periods. Mycorrhiza formation is reduced or lacking in very moist situations as a consequence of low oxygen tension (Gerdemann 1968; Harley and Smith 1983) and this may have contributed to the lack of mycorrhizas on the epiphytes examined in this study. Nadkarni (1981, 1984) reported VAM fungi in canopy root systems and suggested that the fertility of the suspended organic humus affected mycorrhiza formation. Although the available P of the organic debris in which epiphytes were rooted was not determined, the absence of VAM fungi from epiphytes observed in the present study may have resulted from the high fertility of the debris, as well as from the failure of propagules of glomalean fungi to arrive at the positions were epiphytes were growing.

The absence of mycorrhizas from bioassay maize plants might be explained by a lack of inoculum in organic debris. Lesica and Antibus (1990) also failed to produce VAM fungi on onions grown in epiphyte substrates.

The present survey of oil palm rhizosphere soils includes 12 species of glomalean fungi that have only coarse hyphae. The "fine endophyte", *G. tenue*, which has spores less than 12 μm in diameter (Hall 1977) was not recovered from any of the soils sampled. Although attempts were made to root epiphytes in pots containing soils collected from oil palm plantations, only the facultative epiphytes were able to establish in pots and form VAM fungi. The other epiphytes failed to establish, or those that grew did not form any mycorrhizae. Either these species are truly obligate epiphytes or they may need a period longer than 3 months to adapt to soil conditions and form mycorrhizas.

Lesica and Antibus (1990) indicated the need for ecological explanations of the paucity of VAM fungi in epiphytes in view of the fact that many epiphyte species belong to families generally considered mycorrhizal (Gerdemann 1968; Tester et al. 1987). In the present study, it appears that mycorrhizas are not as common or as important on oil palm trunks as in terrestrial soils. Although the epiphytic ferns examined all lacked mycorrhizas, some of their terrestrial conspecifics had VAM fungi. The ability of some mycorrhiza-free transplants of epiphytes to form VAM fungi in the greenhouse complements the occurrence of such fungi in terrestrial plants, indicating that these species can form mycorrhizas but as epiphytes apparently do not.

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