Institut Penyelidikan Perhutanan Malaysia (FRIM) Kepong. 52109 Kuala Lumpur THE ROLE OF MYCORRHIZAS IN THE REGENERATION OF SOME MALAYSIAN LOWLAND RAIN FOREST TREES OF JENGKA

Norani Ahmad

Forest Research Institute Malaysia, Kepong 52109, Kuala Lumpur

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NORANI AHMAD. 1996. The role of mycorrhizas in the regeneration of some Malaysian lowland rain forest trees of Jengka. A survey on the status of mycorrhizal infection was carried out in three representative forest areas of Jengka. Levels of root infection in individual plant species were comparatively high. The assessment also confirmed the taxonomic dominance of vesicular-arbuscular mycorrhiza (VAM) tree species in the Malaysian forest. Sixty species examined were vesicular-arbuscular mycorrhizal and three species were ectomycorrhizal. A significant reduction in percentage mycorrhizal infection was observed in the disturbed forest sites.

Key words: Mycorrhizal infection - taxonomic dominance - vesicular - arbuscular mycorrhiza (VAM)

NORANI AHMAD. 1996. Peranan mikoriza dalam pemulihan sesetengah pokokpokok tanah hutan pamah Malaysia di Jengka. Satu tinjauan telah dijalankan di tiga kawasan yang mewakili kawasan hutan Jengka untuk mengetahui status infeksi mikoriza. Tahap infeksi mikoriza pada akar di spesies tumbuhan individu adalah agak tinggi. Kajian juga mengesahkan bahawa pokok-pokok bermikoriza vasikular-arbuskular adalah taksonomi spesies unggul di hutan Malaysia. Enam puluh spesies yang diselidik adalah bermikoriza vesikular-arbuskular manakala tiga spesies berstatus etomikoriza. Pengurangan peratus infeksi mikoriza yang paling ketara ialah di kawasan-kawasan hutan terganggu.

Introduction

Malaysia has an abundant indigenous forest and although there is considerable silvicultural information on the important tree species, little is known of their mycorrhizas. Mycorrhizas were first reported in Malaysian soils in 1954 by the Rubber Research Institute of Malaysia.

The majority of tropical trees that have been surveyed form vesicular-arbuscular mycorrhizas (VAM) (Janos 1980, Hogberg 1982, Norani 1983, St. John & Uhl 1983, Hogberg & Piearce 1986, Newbery *et al.* 1988) but at least in the paleotropics, the important taxa Dipterocarpaceae and Caesalpinoideae form ectomycorrhizas.

Most past investigations in Malaysia involved ectomycorrhizas of Dipterocarpaceae. Forty-one Malaysian dipterocarp species have been reported to form ectomycorrhizas (Singh 1966, Becker 1983, Lee 1988, Berriman 1986).

The objective of this study is to quantify the type and degree of mycorrhizal infection in some forest tree species and successional species in Jengka.

Materials and methods

Study sites

Three areas were chosen within the Jengka Forest Reserve, to represent an undisturbed forest (site A), a semi-disturbed secondary forest (site B) and logged-over forest (site C).

Site A, an area of minimum disturbance prior to the study, was characterised by absence of stumps and other logging residues and few early successional tree species in the upper canopy. This suggests that previous silvicultural treatment, if any, had had little effect on the overall floristics of the stand.

Site B is in a semi-disturbed forest area where selective logging practices had been carried out before the study commenced. Natural and man-made gaps covered with herbaceous and weedy species were specifically marked for this investigation. Remains of fallen timbers were still present. The soil surface had not been severely disturbed.

Site C had been intensively logged and therefore the stocking differed from the undisturbed conditions in site A. Most of the big trees of merchantable quality had been removed leaving behind trees mostly in diameter size classes 15 - 40 cm dbh. There were ten times more seedlings of non-dipterocarps than dipterocarps and these seedlings formed the major ground cover.

Sampling

Two samplings were carried out at sites A, B and C in August 1985 and in September 1987. Site C had been logged about two years before the study commenced and the later assessment represents a period of approximately five years after logging.

Sampling was carried out systematically by laying out ten 30 m line transects and establishing five 1 m² quadrats at 6 m spacing along each transect at each site. All plants less than 100 cm high within each quadrat were excavated and five plants of each species retained for the assessment of mycorrhizal infection. Where there were less than five, all were retained. A sample of fine roots of plants greater than 10 cm tall within each quadrat were excavated. Plant samples were made into herbarium specimens and identified with the assistance of the Botany Section of the Forest Research Institute Malaysia.

Root assessments

Fine roots were washed free of soil and preserved in formaldehyde-acetic acid (Sass 1958). Roots with fungal mantles were dehydrated and embedded in glycol methacrylate (Alexander & Bigg 1981). Sections 2-5 μ m thick were cut with a glass knife, stained in methylene blue, mounted in balsam and examined. Roots were considered to be ectomycorrhizas when they had a fungal mantle and a Hartig net.

For studies of VA mycorrhizs, the five plants from each species collected were assessed for percentage mycorrhizal infection. Plant roots were cleaned, and either fixed in formalin-acetic acid or cleared immediately using hot KOH (10%). Since the majority of the plant roots were dark colored, they were treated with hydrogen peroxide immediately after clearing with KOH, then washed under running water and stained with methylene blue (Phillips & Hayman 1970). Between 50 and 100 1-cm root segments of each plant were examined and scored for presence or absence of VA infection.

VAM infection was identified by the presence of structures such as arbuscules, intercellular coils, intra- or extra-radical vesicles, coarse aseptate hyphae and fine septate hyphae (Nicolson 1959). Percentage mycorrhizal infection in roots was estimated by the root-slide technique (Daft & Nicolson 1972, Read *et al.* 1976). All infected and uninfected segments were counted and the percentage infection was calculated thus:

% VA infection = Total no. of segments examined × 100

Results and discussion

Floristic composition

In this study it was found that the reproductive habit of plants exerted a strong influence on the establishment of the plant community. Within the forest community, there were differences in species composition between sites A, B and C. The plant community of the disturbed site C was dominated by plants possessing a weedy growth habit or ruderal strategy. Plants from the family Compositae and Gramineae especially, were absent from sites A and B but were predominant in the disturbed site C.

During the three years of study there was very little change in the floristic composition of plant species within each site (Table 1). The plant community in site A, except for seedlings from the canopy species, had remained unchanged and stable.

Although selective logging practices had caused little damage to the soil in site B, the broken canopy cover had encouraged seedlings of pioneer species, shrubs, herbs and climbers to flourish. During September 1987 (year 3), there was an increase in plant density in terms of plant individuals in this site due to survival of early stems coupled with new regrowth. In site C, no seedling species reached high cover or density during August 1985 (year 1) assessment. The presence of herbaceous growth, creepers, ferns, graminoids and trees like *Trema* and *Macaranga* species is evidence of rapid secondary succession.

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| Family | amily Species | | Site A | | Site B | | С | |
|------------------|------------------------|------|--------|------|--------|------|------|--|
| | Year | 1985 | 1987 | 1985 | 1987 | 1985 | 1987 | |
| Adiantaceae | Adiantum latifolium | _ | - | - | - | 0 | 36 | |
| Annonaceae | Friesoc'ielsia glauca | 67 | 73 | - | - | _ | - | |
| | Artabotres sp. | - | _ | 40 | 51 | _ | - | |
| | Monocaroia marainalis | - | - | 35 | 42 | - | - | |
| Aritolochiaceae | Thottea sp. | - | - | 30 | 37 | - | - | |
| Aspidiaceae | Pleocnemia irregularis | - | - | 70 | 78 | - | - | |
| | Tectaria singaporeana | - | - | - | - | 35 | 30 | |
| | Tectaria polymorpha | - | - | - | - | 20 | 31 | |
| Begoniceae | Begonia sp. | - | - | 70 | 75 | - | - | |
| Bombacaceae | Durio zibethinus | - | - | 72 | 63 | - | - | |
| Buseraceae | Santiria sp. | 46 | 79 | 46 | 30 | - | - | |
| Celastraceae | Salacia viminea | 44 | 46 | - | - | - | - | |
| Compositae | Blumea sp. | - | - | - | - | 0 | 15 | |
| | Eupatorium odoratum | - | - | - | - | 25 | 58 | |
| | Mikania cordata | - | - | - | - | 0 | 40 | |
| | Mikania scandens | - | - | - | - | 25 | 58 | |
| Connaraceae | Agelsea macrophylla | 52 | 47 | 45 | 40 | - | - | |
| | Rourea minor | 84 | 79 | - | - | - | - | |
| | Rourea mimosoides | 96 | 81 | - | - | - | - | |
| Dipterocarpaceae | Shorea bracteolata | ** | ** | - | - | - | - | |
| 1 1 | Shorea multiflora | ** | ** | ** | ** | ** | ** | |
| Ebenaceae | Diospyros sp. | - | - | * | * | - | - | |
| Euphorbiaceae | Aporusa sp. | - | | 10 | 20 | - | - | |
| 1 | Cleistanthus maingayi | * | * | - | - | - | - | |
| | Croton argyratus | 98 | 100 | 55 | 47 | - | - | |
| | Elateriospermum tapos | - | - | 50 | 45 | - | - | |
| | Fahrenheitia pendula | - | - | 0 | 28 | - | - | |
| | Macaranga sp. | - | - | - | • _ | 0 | 28 | |
| | Macaranga gigantea | - | ~ | - | - | 10 | 25 | |
| | Macaranga triloba | - | - | - | - | 20 | 34 | |
| | Macaranga conifera | - | - | 40 | 55 | _ | - | |
| | Mallotus sp. | - | ~ | - | - | 12 | 35 | |
| | Sapium discolor | - | - | 12 | 10 | - | - | |
| Gesneriaceae | Didymocarpus sp. | - | - | ** | ** | - | - | |
| Gramineae | Brachiaria paspaloides | - | - | - | - | 55 | * | |
| | Digitaria longiflora | - | - | - | - | 45 | 56 | |
| | Digitaria marginata | - | - | - | - | 15 | 35 | |
| | Imperata cylindrica | - | - | - | - | 35 | 55 | |
| Hypericaceae | Cratoxylum formosum | 96 | 98 | - | - | _ | - | |
| Lauraceae | Actinodaphne pruinosa | - | - | 51 | 47 | - | - | |
| | Lindera lucida | - | - | * | * | - | - | |
| Leeaceae | Leea sp. | - | - | 41 | 46 | - | - | |
| Leguminosae | Adenanthera pavonina | 76 | 82 | - | - | 0 | 40 | |
| 0 | Bauhinia sp. | 100 | 100 | - | - | - | - | |
| | Caesalpinia parviflora | 100 | 100 | - | - | - | - | |
| | Dialiv:m platysepalum | - | - | 20 | 35 | - | - | |
| | *Intsia palembanica | 65 | 72 | - | - | - | - | |
| | Koompassia malaccensis | 78 | 80 | - | - | - | - | |
| | Millettia atropurpurea | - | - | 45 | 60 | - | - | |
| | Mimosa sp. | - | - | - | - | 35 | * | |
| | Parkia javanica | 87 | 98 | - | - | - | - | |
| | Parkia speciosa | 100 | 100 | | | | | |

| Table 1. | Species present at the study sites and the percentage root length infected |
|--------------|--|
| | with VA mycorrhizas |

| Melastomataceae | Clidemia hirta | - | - | 46 | 45 | - | _ |
|------------------|--------------------------|----|----|-----|----|----|----|
| | Pternandra echinata | 48 | 68 | 46 | 45 | 0 | 32 |
| Moraceae | Ficus sp. | 0 | 37 | 0 | 35 | 0 | 18 |
| | Streblus taxoides | * | * | - | - | - | - |
| Myrtaceae | Eugenia sp. | - | - | 70 | 76 | - | - |
| Myrsinaceae | Labisia | - | - | 10 | 22 | - | - |
| Palmae | Daemonorops sp. | 15 | 35 | - | - | - | - |
| | Calamus sp. | 20 | 15 | - | - | - | - |
| Piperaceae | Piper aduncum | - | - | * | * | 46 | 60 |
| Polygalaceae | Xanthophyllum eurhynchum | - | - | * | * | - | - |
| | Xanthophyllum wrayi | - | - | | - | 10 | 12 |
| Rubiaceae | Argostemma ophirense | - | -' | 34 | 25 | - | |
| | Psychotaria sp. | - | - | 15 | 10 | - | - |
| Rutaceae | Citrus macroptera | - | - | - | - | 35 | 50 |
| Thelypteridaceae | Pneumatopteris sp. | - | - | - | - | * | * |
| | Sphaerostephanos sp. | - | - | - | - | * | * |
| Ulmaceae | Trema amboinensis | - | - | - ` | - | 20 | 48 |
| Zingiberaceae | Globba sp. | - | - | - | - | 32 | 35 |

Table 1 (continued)

[Nomenclature according to Whitmore (1972)]

- Species not present in quadrat;

* Missing value due to limited root samples;

** Ectomycorrhizal species.

Type and abundance of mycorrhizas.

The majority of the collection examined was either VAM or ectomycorrhizal. Sixty species were found to be vesicular-arbuscular mycorrhizal and three species were ectomycorrhizal (Table 1). The results confirmed the taxonomic dominance of VA mycorrhizal tree species in the tropics (Janos 1980). Amongst the plant species examined, the roots of *Intsia palembanica* were observed to be infected with either ecto- or vesicular-arbuscular mycorrhiza or sometimes with both infections on the same root portion. It has been established before that a species may have both ectomycorrhizas and VA mycorrhizas simultaneously (Harley & Smith 1983). The ectomycorrhizal status of the Caesalpinioideae has been reported earlier by Alexander (1989).

All roots of VA mycorrhizal plant species sampled contained internal hyphae and hyphal coils. Intra- and extra-radical vesicles and arbuscules were uncommon in the field-collected roots. Arbuscules were perhaps uncommon in most of the roots examined because they are short-lived, seasonal in occurrence, and sensitive to a variety of environmental factors (St. John & Uhl 1983). The mycorrhizal status of these plants can therefore be questioned if one was to accept the suggestion by Herrera *et al.* (1978). He suggested that, since arbuscules are believed to be the major sites of nutrient exchange with the host (Cox *et al.* 1975), the absence of arbuscules could indicate a non-functional mycorrhizal association or a non-symbiotic colonisation of roots by hyphae of nearby mycorrhizal plants. The amount of arbuscular infection would be important in determining if a VA mycorrhiza has formed. In this investigation, mycorrhizal infection was assumed to be functional whether or not arbuscules were seen.

It has been claimed that annuals and weedy species of very disturbed sites are never mycorrhizal (Reeves 1985). However, in this investigation at the time of assessment, such weedy herbaceous species and the grasses had already established the mycorrhizal symbiosis. It may well be that their ruderal strategy helped them through. VAM was not significant then. However, once established, competition begins, and that was when VAM symbiosis was established.

Using the concepts of Grime (1977), secondary succession on heavily disturbed, semiarid soils begins with ruderals (non-mycorrhizal species), then proceeds to competitors (mycorrhizal species), and the "climax" community which is characterised by stress-tolerant (mycorrhizal) species. These concept are supported by similar successional sequence in the tropics (Janos 1980).

Thus in this investigation, following the above concepts, plant species in the disturbed site C and some in site B may be classified as competitors while the majority of the species in site A as stress tolerant species. The interrelationship of stress and disturbance (Grime 1977) determines the type of plants found in an ecosystem. Thus there appears to be a potential correlation between the survival of mycorrhizal fungi and the hosts present on the stress/disturbance matrix (Reeves 1985). If disturbance remains great as in site C, weedy species persist. As disturbance is reduced, succession can proceed either directly to stress-tolerant species (as in site A) or through competitor species to stress-tolerant species (as in site B). In either case, there is the development of plants that are able to host VA mycorrhizal fungi.

Percentage mycorrhizal infection

The mean percentage of mycorrhizal infection in plant species present in sites A, B and C in both periods of mycorrhizal assessments are given in Table 2. Contributing factors for the significant reduction in percentage infection in plant species in sites B and C were probably results of change in species from climax plant community to successional competitors and that land disturbance had reduced mycorrhizal hosts and VAM fungi propagules. The later conclusion is supported by the evidence from plant species that occurred both in sites A and B, e.g. *Santiriasp., Agelsea macrophylla* and *Croton argyratus*, or in all three sites, e.g. *Pternandra echinata* and *Ficus* sp.

Generally, percentages of infection in the same species in different sites were lower in the semi-disturbed sites B and C as compared to the undisturbed site A.

In each site the percentage infections in year 1 and year 3 were compared using paired *t*-test. The increased infection in year 3 was significant only in site C (p < 0.05). All data were subjected to Arcsine transformation prior to analysis.

| | Site | No. of species examined | Year 1 | Year 3 - | |
|---|----------------|----------------------------|--------|---------------|--|
| А | Undisturbed | 19 | 73.5a* | 67.4a | |
| В | Disturbed | 25 | 38.1b | 42.7b | |
| С | Very disturbed | 24 | 19.8c | 34.6 d | |

Table 2. Percentage mycorrhizal infection in plant species from the undisturbed site A,
disturbed site B and the very disturbed site C

* Any two means having a common letter are not significantly different at 5% level (One way Analysis of Variance F< p).

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References

- ALEXANDER, I.J. 1989. Systematics and ecology of ectomycorrhizal. Pp. 607-627 in Stirton, C.H. & Zarucchi, J.L. (Eds). Advances in Legume Biology. Monograph Systematic Botanical, Missouri Botanical Garden 29.
- ALEXANDER, I. J. & BIGG, W.L. 1981. Light microscopy of ectomycorrhizas using glycol metacrylate. Transactions of the British Mycological Society 77: 428.
- BECKER, P. 1983. Ectomycorrhizae on *Shorea* (Dipterocarpaceae) seedlings in a lowland Malaysian rain forest. *Malaysian Forester* 46 : 146 -170.
- BERRIMAN, C.P. 1986. Mycorrhizas of *Shorea* (Dipterocarpaceae) in relation to host specificity and soil phosphorus status. B.Sc. (Hons.) thesis, University of Aberdeen.
- COX, G., SANDERS, F.E., TINKER, P.B. & WILD, J. A. 1975. Ultrastructural evidence relating to hostendophyte transfer in a vesicular-arbuscular mycorrhiza. Pp. 297-312 in Sanders, B., Mosse, B. & Tinker, P.B. (Eds.) *Endomycorrhizas*. Academic Press, London and New York.
- DAFT, M.J. & NICOLSON, T.H. 1972. Effect of *Endogone* mycorrhiza on plant growth. IV. Quantitative relationship between the growth of host and development of endophyte in tomato and maize. *New Phytologist* 73 : 1129 - 1138.
- GRIME, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* 111: 1169 1174.
- HARLEY, J. L. & SMITH, S.E. 1983. Mycorrhizal Symbiosis. Academic Press, London. 483 pp.
- HERRERA, R., MERIDA, T., STARK, N. & JORDANA, C.R. 1978. Direct phosphorus transfer from leaf litter to roots. *Naturwissenschaften* 65: 208-209.
- HOGBERG, P. 1982. Mycorrhizal associations in some woodland and forest trees and shrubs in Tanzania. New Phytologist 92: 407-415.
- HOGBERG, P. & PIEARCE, G. D. 1986. Mycorrhizas in Zambian trees in relation to host taxonomy, vegetation communities and successional patterns. *Journal of Ecology* 74: 775-785.
- JANOS, D.P. 1980. Vesicular-arbuscular mycorrhizae affect lowland tropical rain forest plant growth. Ecology 61(1): 151-162.
- LEE, S.S. 1988. The ectomycorrhizas of Shorea leprosula. Pp. 189-206 in Ng, F.S.P. (Ed.) Proceedings of the Asian Seminar on Trees and Mycorrhizas. April 1987, Kuala Lumpur.
- NEWBERRY, D.M., ALEXANDER, I.J. & THOMAS, D.W. 1988. Ectomycorrhizal rain forest legumes and soil phosphorus in Korup National Park, Cameroon. *New Phytologist* 109: 433-450.

NICOLSON, T. H. 1959. Mycorrhiza in the Gramineae. I. Vesicular-arbuscular endophytes, with special reference to the external phase. *Transactions of the British Mycological Society* 42:421-438.

- NORANI, A. 1983. A preliminary survey on nodulation and VA mycorrhiza in legume roots. *Malaysian* Forester 46 (2): 171 - 174.
- PHILLIPS, J. H. & HAYMAN, D.S. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55: 158 - 161.
- READ, D. J., KOUCHEKI, H.K. & HODCSON. 1976. Vesicular-arbuscular mycorrhizae in natural vegetation ecosystems. *New Phytologist* 77: 641-653.
- REEVES, F. B. 1985. Survival of VA mycorrhizal fungi-interactions of secondary succession, mycorrhizal dependency in plants, and resource competition. Pp. 110-113 in Molina, R. (Ed.) *Proceedings of the 6th North American Conference on Mycorrhizae.* Forest Research Laboratory, Oregon State University, Corvallis.
- RUBBER RESEARCH INSTITUTE of MALAYA. 1954. Mycorrhiza in Hevea. Planters Bulletin, Rubber Research Institute Malaya 12:57-58.

SASS, J. E. 1958. Botanical Microtechnique. 3rd. edition Iowa State College Press, Ames, IA.

SINGH, K.G. 1966. Ectotrophic mycorrhizae in equatorial rain forest. *Malayan Forester* 29:13-18. St. JOHN, T.V. & UHL, C. 1983. Mycorrhizae in the rain forest at San Carlos de Rio Negro, Venezuela.

Acta Scientia Venezolana 34 : 233 - 237.

WHITMORE, T.C. 1972. (Ed.) Tree Flora of Malaya. Volume 1. Longman Malaysia, Kuala Lumpur.