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The role of mycorrhizas in the regeneration of some Malaysian forest trees

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SUMMARY

An investigation was made into the availability of mycorrhizal inoculum and the response of tree seedlings to mycorrhizal infection in West Malaysian forests. Spores of vesicular arbuscular (VA) mycorrhizal fungi in the soil were reduced by 25% after selective logging and by 75% after heavy logging. VA infection in the roots of plants persisting on, or colonizing, a heavily logged site was reduced by up to 75%. The most probable number (MPN) of VA propagules in sieved soil was up to ten times greater than spore density, but was also greatly reduced by heavy logging. This resulted in reduced infectivity of soil from the heavily logged site, as demonstrated by reduced VA infection of bioassay plants. The infectivity of soil declined following sun drying, but sun-dried soil devoid of vegetation retained some infectivity even after 12 months storage. Overall the data suggest that root and hyphal fragments are more important than spores as inoculum in disturbed forest, and that in undisturbed forest living roots and hyphae are likely to be important sources of infection.

In a pot experiment, shoot growth of two test species, *Albizia falcataria* (L.) Becker and *Parkia speciosa* Hassk. responded more to VA mycorrhizal infection than to P fertilization over the range 0–6 g triple superphosphate per 8 kg of soil. The response to inoculation with a cocktail of 'introduced' VA fungi propagated in pot cultures was greater than the response to inoculation with 'indigenous' fungi propagated in pot cultures from roots and soil collected in undisturbed forests. Another test species, *Intsia palembanica* Miq., also responded better to mycorrhizal infection than to P fertilization, and better to VA mycorrhizal infection than to ectomycorrhizal infection.

Intsia palembanica seedlings growing around mature dipterocarps quickly became ectomycorrhizal, suggesting that at least some ectomycorrhizal fungi infect both dipterocarps and *Intsia*. *Shorea leprosula* Miq. seedlings growing naturally in the forest had ectomycorrhizas 20 days after germination, i.e. before they had true leaves, and within 7 months supported up to 11 different ectomycorrhizal fungi. However, seedlings isolated from contact with the roots of mature *Shorea* trees remained uninfected in the field for up to 6 months. This shows the importance of contact with living ectomycorrhizal roots for early infection of dipterocarp seedlings, a point which should be recognized in logging operations and forest regeneration programmes.

1. INTRODUCTION

Most tropical trees are habitually mycorrhizal in natural soils. Their fine roots are infected by symbiotic fungi which derive carbohydrate from the plant and which, in turn, are assumed to confer some benefit on the host. The benefit is thought to result largely from improved access to soil nutrients and soil water, or from protection against pathogenic microorganisms. Most experiments that demonstrate benefit, i.e. improved growth or survival of the host, resulting from mycorrhizal infection, have been done in laboratory conditions or in pot trials, and real tests of mycorrhizal benefit in field situations are uncommon (Fitter 1985, 1986). Nevertheless, there is a wealth of circumstantial evidence to suggest that the extent and nature of mycorrhizal infection might be an important factor determining the survival and growth of tropical tree seedlings, and thus the regeneration and recovery of tropical rain forest after disturbance.

Most tropical tree taxa from vesicular arbuscular (VA) mycorrhizas. The fungus penetrates the root intercellularly and intracellularly, and gives rise to characteristic hyphal coils, vesicles and arbuscules. A loose weft of fungal hyphae extends out from the root into the substrate. These hyphae may bear spores which form the basis of the taxonomy of VA mycorrhizal fungi. Currently, about 140 species are recognized (Morton & Benny 1990) in six zygomycetous genera. Many have a cosmopolitan distribution, and they display little host specificity (but see McGonigle & Fitter 1990). In contrast, an ecologically and economically important minority of tropical tree taxa form ectomycorrhizas (Janos 1983; Alexander 1989). In South-east Asian forest this includes all Dipterocarpaceae, Fagaceae and Myrtaceae sf. Leptospermoideae

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as well as *Intsia* spp. (Leguminosae sf. Caesalpinoideae). Ectomycorrhizas differ structurally from VA mycorrhizas in that fungal tissue forms a sheath around the host root and penetrates between root epidermal cells to form a labyrinthine structure, the Hartig net. From the sheath surface, not only individual hyphae but also complex hyphal strand systems radiate out into the substrate. Ectomycorrhizas are formed predominantly by basidiomycete and ascomycete fungi (see, for example, Hong (1979); Thoen & Ba (1989)) and host specificity, i.e. the restriction of a fungus to one or a few related host taxa, is common (Molina & Trappe 1982), although many broad host-range fungi also occur.

A detailed comparison of the role of VA mycorrhizas and ectomycorrhizas in nutrient and water uptake is beyond the scope of this paper, and the reader is referred to recent reviews (Harley & Smith 1983; Alexander 1989; Safir 1987). In summary, both types of mycorrhizal infection are thought to increase the inflow of immobile elements, such as phosphorus, to the root. There is increasing evidence that ectomycorrhizas may utilize organic sources of nitrogen and phosphorus. In addition, the sheath of ectomycorrhizas has important storage functions which may be of particular advantage where nutrient availability is intermittent, e.g. in seasonal climates. Read (1991) has suggested that the functional attributes of VA mycorrhizas are consistent with their predominance in ecosystems where P availability is the major nutritional limitation to plant growth, whereas the attributes of ectomycorrhizas are consistent with ecosystems where organic matter accumulates at the soil surface and N availability limits growth. This conclusion relies heavily on the results of experiments with northern temperate and boreal species, and comparable work on tropical tree mycorrhizas has not been done.

To derive the benefits of mycorrhizal infection, newly germinated tree seedlings must become infected. There are three potential sources of inoculum: spores, living hyphae attached to living roots, and detached hyphae or vesicles in soil or in dead root fragments. Knowledge of the relative importance of these sources of inoculum in undisturbed forest, and the extent to which forest disturbance affects them, is important to an understanding of the role of mycorrhizas in forest recovery. It is known, for example, that both ectomycorrhizal and VA mycorrhizal plants of the same or different species can be interconnected by hyphal bridges (Read 1989), and that export of assimilates can occur from canopy trees to shaded seedlings (Read *et al.* 1985). Mycorrhizal infection can alter the outcome of interspecific competition in microcosms (Janos 1983, 1985; Grime *et al.* 1987), and the movement of nutrients and assimilate through a shared mycelial network may contribute to this effect.

Different species respond differently to mycorrhizal infection (Janos 1980). For example, some show a high degree of 'dependence', i.e. they do not grow in natural soils unless infected; others are highly 'responsive', i.e. they can grow in the absence of infection but show greatly increased growth when infected. Again, a full discussion of the relationship between the mycorrhizal response and other factors, such as root architecture and nutrient availability, is outwith the scope of this paper. However, it is clear that the interactions between inoculum availability and differing degrees of mycorrhizal dependence or responsiveness could greatly affect forest regeneration processes.

In this paper we present data from a series of investigations done in West Malaysia into aspects of inoculum availability and seedling response. These investigations fall into three groups.

1. A study of VA mycorrhizal inoculum and how it is affected by logging practice.
2. A study of the response to mycorrhizal infection of seedlings of three contrasting legume species.
3. A study of the availability of ectomycorrhizal inoculum for *Intsia* and *Shorea* spp. and the timecourse of infection of naturally regenerated *Shorea* spp. seedlings in the field.

2. METHODS AND MATERIALS

(*a*) *VA mycorrhizal inoculum*

(i) *The study site*

Three areas of uniform topography on Batu Anam series soils were chosen in 1985 within the Red Meranti-Keruing (Wyatt-Smith 1961) mixed dipterocarp forest of Jengka Forest Reserve, Malaysia. The first was an area of undisturbed forest. The second was an area which had been selectively logged, leaving frequent gaps in the forest cover. The third area had been logged in 1983 and all trees of at least 40 cm dbh had been removed. There had been extensive soil disturbance in this area: some parts were heavily compacted, and in other parts the topsoil had been removed and there was considerable erosion.

(ii) *Spore numbers*

Composite samples (30), each consisting of five 5.0 cm diameter cores, were taken at random from the top 15 cm of soil in each area in June 1985 and again in January 1986. These are the periods of maximum and minimum rainfall in Jengka. The samples were air-dried and thoroughly mixed before the spores of VA mycorrhizal fungi in a 100 g subsample were extracted by wet sieving and sucrose centrifugation (Walker *et al.* 1982). Live spores were counted under $\times 60$ magnification.

(iii) *Levels of mycorrhizal infection*

Ten 30 m line transects were laid out at random in each area in August 1985 and September 1987. Every 6 m, a 1 m^2 quadrat was established and all the plants rooted within the quadrats were identified. The root systems of the first five individuals less than 100 cm high of each species encountered were excavated. For plants greater than 100 cm high a sample of fine roots was excavated. The roots were cut into 1 cm segments, and between 50 and 100 segments from each plant were cleared and stained (Phillips & Hayman 1970). VA mycorrhizal infection was expressed as the percent-

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age of segments of each species which showed arbuscules, or intracellular coils or coarse intracellular hyphae.

Because the roots of the large woody vegetation were under-represented by this sampling procedure, the roots retained on the 600 μm sieve used in the initial step of spore extraction (see above) were also cut into 1 cm segments and scored for VA mycorrhizal infection.

(iv) *Infectivity of soils*

The most probable number (MPN) of infective propagules (i.e. spores, hyphae or root fragments) in soils from the three areas was estimated following the method of Porter (1979). For this exercise the extensively logged area was stratified into three parts, those areas where topsoil had been removed, those where topsoil had been compacted, and the remainder. From each area, 100 blocks of soil 30 cm square and 15 cm deep were collected at random, bulked, mixed and passed through a 2 mm sieve. A twofold dilution series was prepared by mixing 100 g of fresh sieved inoculum soil with 100 g of steam-sterilized diluent soil. This mixture was then successively diluted with steam-sterilized soil until a 2^{-7} dilution was achieved. Steam-sterilized soil (3 kg), to which 0.2 g of $(NH_4)_2SO_4$ and 0.045 g muriate of potash had been added, was placed in pots of diameter 18 cm. A 2 cm diameter core was removed from the centre of each pot, 10 g of diluted soil was inserted, and the hole was topped up with steam-sterilized soil. Each dilution of each test soil was replicated five times, and there were five control plots without diluted soil. Seeds of *Albizia falcataria* (L.) Becker were germinated in sterile sand and planted in the pots. After 8 weeks the root systems of the seedlings were cleared and stained and scored for presence or absence of VA mycorrhizal infection. These data were used to derive the MPN of infective propagules by reference to table $VIII_2$ of Fisher & Yates (1963).

Another estimate of the relative infectivity of soils from the three original areas was obtained by growing seedlings in pots of the soil and following the time-course of mycorrhizal infection. The species used were *A. falcataria* and *Intsia palembanica* Miq. Soil from the top 10 cm of the profile was used to fill pots of diameter 18 cm. No fertilizer was added, and steam-sterilized soil supplemented with 200 ml of microbial suspension from non-sterilized soils served as a control against non-soil-derived infection. Pre-germinated seedlings were introduced into the pots and grown in a shade house. Plants were harvested 1, 2 and 4 months later, and percentage mycorrhizal infection estimated as before.

Because, after mechanized logging, areas of soil can remain devoid of vegetation for some time, an investigation was done into the persistence of VA mycorrhizal propagules. Soil was collected from the top 30 cm of the profile in the undisturbed forest at Jengka, passed through a 2 mm sieve, and divided into three equal portions. One portion was moistened and immediately sealed into polythene bags. A second portion was air-dried in direct sunlight for 10 h every day for 2 weeks, then moistened and sealed into polythene bags. The third portion was steam-sterilized at 100°C for 2 h, moistened, and bagged. Before bagging, a sample of each soil was removed, put in pots, and seeded with *Allium ascolonicum*. The bagged soils were stored at 23°C, and after 3, 6, 9 and 12 months, samples were removed and again seeded with *A. ascolonicum*. On each occasion the plants were grown for 6 weeks and percentage mycorrhizal infection assessed as before.

(*b*) *Growth response to mycorrhizal infection*

(i) *The test species*

Three contrasting legume species were chosen to examine seedling response to infection. *Albizia falcataria* (Mimosoideae) is naturalized in Malaysia and is used in plantation forestry. It has a small seed (6 mm) and a finely divided, sparsely nodulated, seedling root system. *Parkia speciosa* Hassk. (Mimosoideae) has larger seeds (15 mm) and the seedling root system, although still highly branched and nodulated, is somewhat coarser than that of *Albizia*. *Intsia palembanica* (Caesalpinoideae) has a large seed (50 mm) and makes rapid initial growth on the basis of cotyledon reserves (Sasaki & Ng 1981). It has a coarse sparingly branched seedling root system and is not nodulated. *Intsia* is a genus which can form either VA mycorrhizas or ectomycorrhizas. Adult trees in the forest are ectomycorrhizal.

(ii) *The experimental procedure*

A pot experiment was designed: (i) to examine the relative response of the three species to mycorrhizal infection; (ii) to see whether, as in temperate herbaceous species, the mycorrhizal response could be replaced by phosphorus fertilization; and (iii) to compare the response to infection by indigenous VA mycorrhizal fungi with the response to infection by a mixture of introduced species. This last approach was prompted by the suggestion (Fitter 1985) that indigenous strains of VA fungi may be ineffective in nutrient capture.

The potting medium was a 3:1 mixture of forest soil and coarse sand. This was steam-sterilized at 100°C for 1 h and allowed to stand for 2 weeks before use. Each pot contained 8 kg of substrate into which 1.25 g urea and 2.65 g muriate of potash were incorporated. Triple superphosphate (TSP) was added at the rate of 0, 1, 2, 4 and 6 g per pot. VA mycorrhizal inoculum was introduced as a layer 13 cm below the soil surface consisting of 100 g fresh mass of chopped roots of *Setaria anceps* var. *splendida* from pot cultures (Schenk 1982). These plants had been propagated in sterilized soil inoculated with either roots and soil from undisturbed forest ('indigenous inoculum') or a mixture of equal portions of roots from previous pot cultures of *Setaria* inoculated with *Acaulospora laevis* Gerd. & Trappe, *Glomus monosporum* Gerd & Trappe, *Gigaspora calospora* (Nicol. & Berd.) Gerd & Trappe, *Glomus mosseae* Gard & Trappe, and *Glomus fasciculatum* (Thaxter *sensu* Gerd.) Gerd. & Trappe ('introduced inoculum'). The non-mycorrhizal control treatment

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received 100 g of chopped sterilized *Setaria* roots and 25 ml of a filtered soil suspension.

For *A. falcataria* and *P. speciosa* a comparison was made between 'indigenous' and 'introduced' VA mycorrhizal inoculum. For *I. palembanica* the comparison was between 'indigenous' VA mycorrhizal inoculum and ectomycorrhizal inoculum. The latter consisted of 100 g fresh mass of chopped ectomycorrhizal dipterocarp roots. Single pre-germinated seeds were introduced into the pots and the seedlings were grown in a shade house at the Forest Research Institute of Malaysia, Kepong, for 7 months under ambient conditions. Shoot dry mass (70°C) was recorded and VA mycorrhizal infection assessed as before. Ectomycorrhizal infection was assessed by counting the percentage of total short root tips per plant converted to mycorrhizas.

(iii) *Ectomycorrhizal infection of Intsia and Shorea*

Although *Shorea* spp. and *Intsia palembanica* are among the most important timber trees in Malaysia, and *Shorea* spp. are of immense ecological importance, very little is known about how and when their seedlings become ectomycorrhizal. Three simple experiments were done to determine: (i) if ectomycorrhizal fungi of dipterocarps are able to infect *Intsia*; (ii) when *Shorea* seedlings become ectomycorrhizal; and (iii) the importance of living roots as a source of inoculum for *Shorea* seedlings.

The Dipterocarp Arboretum at the Forest Research Institute of Malaysia has, to one side, an area of mown grass. A large specimen of *Dipterocarpus costulata* was selected at the edge of the Arboretum, and seeds of *Intsia palembanica* were sown in concentric semicircles 5, 10, 20, 30, 40 and 50 m from the tree base in the mown grass. At 1, 2, 3 and 4 months after planting, four seedlings were harvested at each distance to determine dry mass of shoots and percentage of short root tips converted to ectomycorrhizas.

Seeds of *Shorea leprosula* Miq. were sown beneath parent trees in secondary forest at two sites in Gombak Forest Reserve and one in Ulu Langat Forest Reserve. Germinating seedlings were numbered, and 15 randomly chosen seedlings were excavated at each site after 20 days and then each month for 7 months after germination. The root systems were examined microscopically and the number of different ectomycorrhizal fungal associations present recorded following the methods of Agerer (1986) and Ingleby *et al.* (1990).

Table 1. *Mean (± s.e.) number of spores of VA mycorrhizal fungi recovered from the top 15 cm of soil at three sites in Jengka Forest Reserve (n = 30)*

(Values followed by the same letter are not significantly different ($p \leqslant 0.05$).)

	number of spores per 100 g air-dry soil	
	January 1985	August 1986
undisturbed	25.3 ± 2.0 a	24.5 ± 1.8 a
selectively logged	31.9 ± 3.4 b	31.5 ± 3.0 b
heavily logged	6.1 ± 0.6 c	8.4 ± 0.8 c

PVC tubes (30), each 30 cm long and 11 cm in diameter, were inserted in the soil under each of three *S. leprosula* trees in secondary forest at Semenyih Forest Reserve to isolate soil from root contact. After 3 weeks, seeds of *S. leprosula* were sown inside and outside the tubes. Germinating seedlings were numbered, and each month for 6 months ten randomly chosen seedlings were excavated, five inside and five outside the tubes, under each tree. The root systems were examined for the presence or absence of ectomycorrhizas.

3. RESULTS

(a) *VA mycorrhizal inoculum*

(i) *Spore numbers*

Spore numbers were low (table 1). The greatest number were found in the selectively logged site. The numbers in the heavily logged site were less than 35% of those in undisturbed forest. There was no difference in spore number at the two sampling dates.

(ii) *Level of mycorrhizal infection*

In the line transect survey, 70 plant species in 31 families were encountered. All were infected with VA mycorrhizal fungi except *Shorea* spp., which had ectomycorrhizas. A summary of the levels of VA mycorrhizal infection is given in table 2: records for individual species are given in Ahmad (1989). Mean root length infected was lower in the selectively logged site than in undisturbed forest, and lowest in the heavily logged site. There was no effect of sampling date in undisturbed and selectively logged forest but in the heavily logged site infection was greater in 1987 (4 years after logging) than in 1983 (2 years after logging). Because different host plant species were present at each site, it is not possible from the above to say to what extent the decline in mycorrhizal infection following disturbance results from changes in the composition of the vegetation as opposed to effects on inoculum availability and infection dynamics, However, five species occurred in both the undisturbed and selectively logged areas, and their mean level of infection was reduced in the latter (table 3). Two species, *Pternandra*

Table 2. *Mean percentage root length infected with VA mycorrhizal fungi of plant species in three sites in Jengka Forest Reserve*

(Values in the same column with the same letter are not significantly different ($p \leqslant 0.5$, one-way ANOVA). Values in the same row with the same letter are not significantly different (paired t-test, $p \leqslant 0.05$). All data were subjected to Arcsin transformation before statistical analysis.)

	number of species examined	August 1985	September 1987
undisturbed	19	73.5 a	67.4 a
selectively logged	25	38.1 b	42.7 b
heavily logged	22	19.8 c	34.6 d

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Table 3. *Mean percentage root length infected with* VA *mycorrhizal fungi for five species which occurred on transects in both undisturbed and selectively logged forest in Jengka Forest Reserve; the species were Santiria sp., Agelsea macrophylla, Croton argyratus, Pternandra echinate, and Ficus sp.*

(Values followed by the same letter are not significantly different ($p \leqslant 0.05$, two-way ANOVA, Arcsin transformation).)

	August 1985	September 1987
undisturbed	48.4 a	66.2 a
selectively logged	38.4 b	39.4 b

Table 4. *Mean (* $\pm$ *s.e.) percentage root length infected with* VA *mycorrhizal fungi of roots from random cores of the top 15 cm of soil at three sites in Jengka Forest Reserve (*$n = 30$*)*

(Values with the same letter are not significantly different ($p \leqslant 0.05$, two-way ANOVA, Arcsin transformation).)

	January 1985	August 1986
undisturbed	77.6 $\pm$ 2.7 a	72.9 $\pm$ 2.6 a
selectively logged	77.5 $\pm$ 2.6 a	76.1 $\pm$ 2.5 a
heavily logged	42.5 $\pm$ 1.5 b	46.8 $\pm$ 1.3 b

Table 5. *The most probable number (*MPN*) of* VA *mycorrhizal propagules in sieved soil from the top 15 cm of the profile at three sites in Jengka Forest Reserve (*$n = 5$*)*

(Values followed by the same letter are not significantly different ($p \leqslant 0.05$, Duncan's Multiple Range Test). Control plants grown in steam-sterilized soil remained uninfected throughout the experiment.)

	MPN propagules per 100 g fresh soil ($\pm$ s.e.)
undisturbed	307.5 $\pm$ 13.8 a
selectively logged	291.2 $\pm$ 7.8 a
heavily logged	75.3 $\pm$ 2.6 b
heavily logged (compacted)	28.0 $\pm$ 1.6 c
heavily logged (eroded)	6.0 $\pm$ 1.5 d

echinata and *Ficus* sp., occurred in both selectively and heavily logged areas: their mean infection was 50–60% lower in the latter.

Mean root length infected in the random cores was significantly lower in the heavily logged site than in the undisturbed or selectively logged sites (table 4). Overall the levels of infection in these samples, which were thought better to represent the woody vegetation, were higher, particularly in the logged sites, than those of the seedling and herbaceous vegetation.

(iii) *Infectivity of soils*

The MPN of VA mycorrhizal propagules in the heavily logged site was *ca.* 25% of that in the undisturbed and selectively logged forest (table 5). Where the heavily logged site had been compacted, or the topsoil eroded, MPN was lower still.

Seedlings of *A. falcataria* grown in soils from the three sites were infected 1 month after germination. The level of infection was greatest in soil from the undisturbed site and least in soil from the heavily logged site (figure 1*a*). This difference was maintained over the 2 month and 4 month harvests. At 4 months the level of infection is soil from the undisturbed site (80%) was twice that in soil from the heavily logged site (40%). *I. palembanica* seedlings were not infected at the first harvest. This is normal in these experimental conditions (Ahmad 1989) and presumably reflects the fact that the first month's growth is made on cotyledon reserves. Thereafter the pattern of infection was similar to *A. falcataria* but the final levels at 4 months were 25% lower. Control plants grown in steam-sterilized soil remained uninfected through the study period.

The infectivity of soil from the undisturbed forest, measured as percentage root length infected of *Allium ascolonicum*, declined by 50% over 12 months' storage at 23°C (figure 2). However, the soil was still infective at the end of the storage period. An initial period of sun drying reduced infectivity by 40% but thereafter the rate of decline was similar to that in the soil stored fresh. Control plants grown in stored steam-sterilized soil never became infected.

(*b*) *Growth response to mycorrhizal infection*

The 'indigenous' and 'introduced' VA mycorrhizal inoculum gave rise to similar levels of infection in the roots of *A. falcataria* and *P. speciosa* after 7 months (figure 3*a*, *b*). Infection declined in both species with increasing amounts of P applied to the soil. The decline was more marked in *A. falcataria*, but 30% of root length remained infected even at the highest level of P application. Control plants which did not receive inoculum remained uninfected throughout the experiment. In contrast, the 'introduced' inoculum invoked a much greater shoot growth response in these two species than did the 'indigenous' inoculum, at all levels of P supplied (figure 4*a*, *b*). This was particularly marked for *P. speciosa*, where the response ((dry mass (DM) of mycorrhizal plant − DM of non-mycorrhizal plant)/DM of non-mycorrhizal plant × 100) was 325% for 'introduced' inoculum but only 25% for 'indigenous' inoculum. The equivalent values for *A. falcataria* were 170% and 90%.

The response to P application by *A. falcataria* and *P. speciosa* was much less than the response to 'introduced' VA inoculum, and even at the highest rates of P application mycorrhizal plants maintained their differential over non-mycorrhizal plants despite the decline in root length infected (figures 3 and 4). There was some evidence that the shoot growth of plants given 'introduced' inoculum declined above applications of 4 g TSP per pot.

'Indigenous' VA mycorrhizal inoculum gave rise to a similar level of infection in *I. palembanica* as in the other two species (figure 3*c*). Infection declined with

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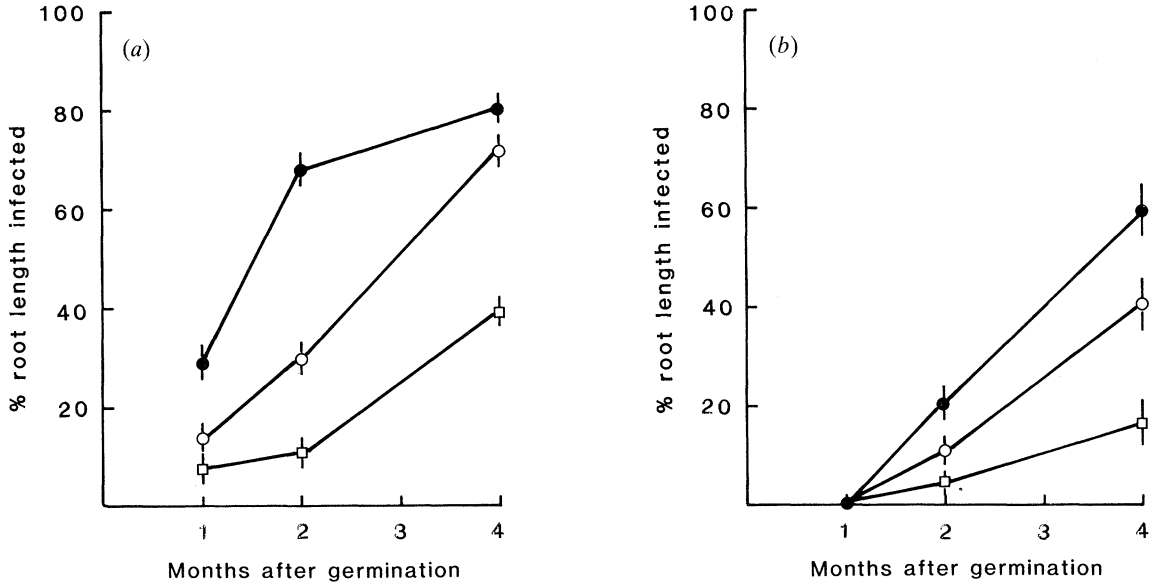

Figure 1. Timecourse of VA mycorrhizal infection of seedlings of (*a*) *Albizia falcataria* and (*b*) *Intsia palembanica* grown in sieved soil from undisturbed (filled circles), selectively logged (open circles) and heavily logged (open squares) areas in Jengka Forest Reserve. Each point is the mean of ten seedlings; bars are 95% confidence limits.

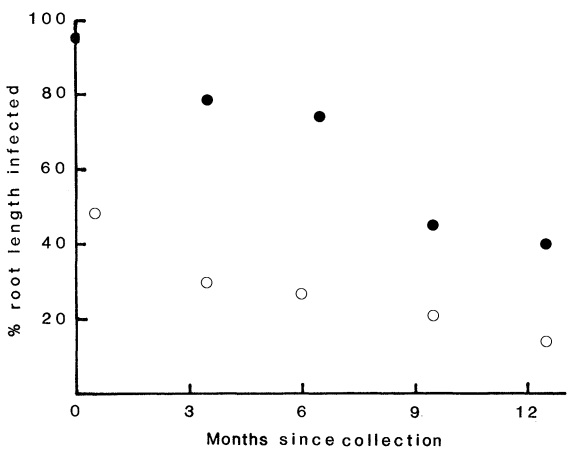

Figure 2. Decline in infectivity of fresh (filled circles) and sun-dried (open circles) soil from undisturbed forest at Jengka during storage, measured as percentage root length infected by VA mycorrhiza of *Allium ascolonicum*. Each point is the mean of five replicate samples.

increasing P application. From 65–80% of the short roots were converted to ectomycorrhizas in the pots given ectomycorrhizal inoculum: there was a slight decline in infection with increasing P application. Shoot dry mass response with VA mycorrhizal inoculum was 100–120%, and with ectomycorrhizal inoculum was significantly lower at 20–50% (figure 4*c*). The mycorrhizal response was greater than the response to P application (figure 4*c*). The mycorrhizal plants had a linear response to P application up to 4 g triple superphosphate per pot, but declined markedly thereafter. The non-mycorrhizal plants had a flatter response but also declined above 4 g per pot.

(*c*) *Ectomycorrhizal infection of* Intsia *and* Shorea

All the *I. palembanica* seedlings at the edge of the Dipterocarp Arboretum became infected with ectomycorrhizas within one month (figure 5*a*). The level of infection increased over the 4 month period of the experiments. Infection declined markedly at distances greater than 30 m from the *Dipterocarpus costulata* tree. At the second, third and fourth harvests, shoot dry mass was positively correlated with percent mycorrhizal infection (figure 5*b*).

Some root tips on 90% of the *Shorea leprosula* seedlings sampled 20 days after germination, i.e. before the first true leaves had expanded and while green cotyledons were still attached, had well-developed fungal sheaths but no Hartig net. However, one month after germination over 70% of seedlings had fully developed mycorrhizas (figure 6*a*). At subsequent harvests 85–100% of seedlings were infected. The number of taxa of ectomycorrhizal fungi present on the seedling roots increased over the sampling period (figure 6*b*).

None of the seedlings inside the tubes had ectomycorrhizas one month after germination, whereas only two seedlings outside the tubes remained uninfected. Over the subsequent 5 months the mean number of infected seedlings within the tubes ranged from two to four (out of a possible five), whereas only one uninfected seedling was found outside the tubes; 6 months after germination 50% of the seedlings within the tubes remained uninfected.

4. DISCUSSION

(*a*) *Effects of logging on* VA *inoculum*

The number of VA spores in the undisturbed forest was low compared with some estimates from South-

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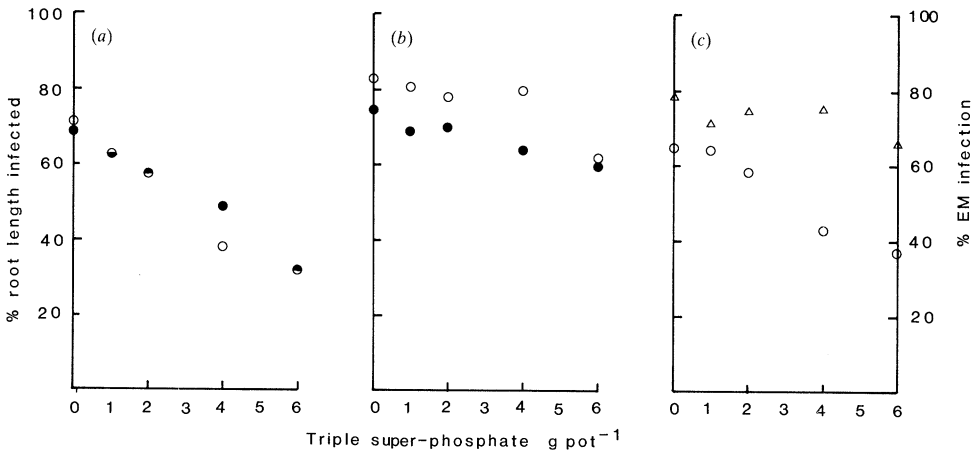

Figure 3. The effect of triple superphosphate application on mycorrhizal infection of (*a*) *Albizia falcataria*, (*b*) *Parkia speciosa* and (*c*) *Intsia palembanica*, grown for 7 months in steam-sterilized soil inoculated with 'indigenous' VA inoculum (open circles), 'introduced' VA inoculum (not *Intsia*) (filled circles) or ectomycorrhizal inoculum (*Intsia* only) (open triangles) ($n=4$). Non-inoculated plants remained free of infection.

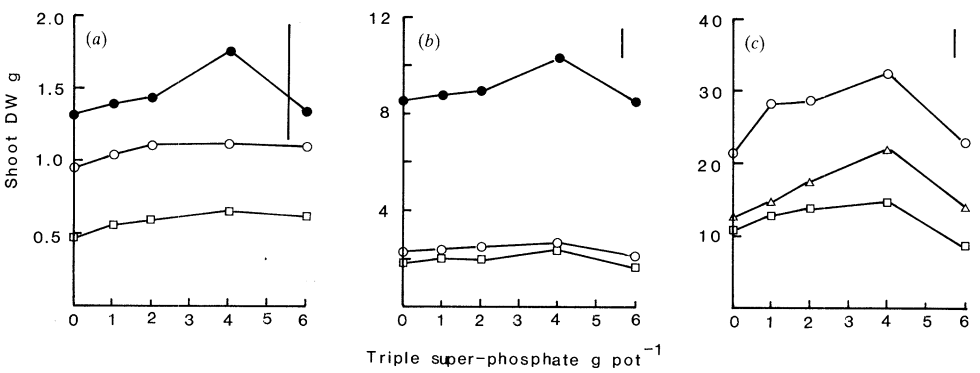

Figure 4. Response to mycorrhizal infection (shoot dry mass) of (*a*) *Albizia falcataria*, (*b*) *Parkia speciosa* and (*c*) *Intsia palembanica*, grown in steam-sterilized soil for 7 months at six levels of triple superphosphate application in the presence of 'indigenous' VA inoculum (open circles), 'introduced' VA inoculum (not *Intsia*) (closed circles), ectomycorrhizal inoculum (*Intsia* only) (open triangles), or un-inoculated (open squares) ($n=4$). Bars are pooled Least Significant Difference for comparison between inocula at a given level of P application.

east Asian soils (see, for example, Chulan (1986)), but within the range encountered in natural vegetation elsewhere (see, for example, Jasper *et al.* (1991)). The selectively logged site had *ca.* 25% more spores but the heavily logged site had 75% less than the undisturbed forest. Loss of vegetation cover and changes in soil conditions are likely to be the cause of the decline. Although spore numbers were low, the level of VA infection of roots in the undisturbed forest was high (*ca.* 70% root length infected), as has been shown previously (Janos 1983; Alexander 1989). Selective logging reduced infection in the smaller plants by up to 50%, but infection of the larger woody plants was probably unaffected. The reduction in the smaller plants was not solely due to colonization by species less prone to infection. The plants persisting on, or colonizing, the heavily logged site had 35–75% less infection. These data are important in that they describe the inoculum environment in which germinating seedlings develop. The high levels of VA infection in undisturbed or selectively logged forest are likely to support extensive mycelial networks which, in natural vegetation, are considered to be the primary source of infection (Read 1989). In contrast, on the heavily logged site all three sources of inoculum (mycorrhizal roots, hyphae and spores) were reduced, and this is likely to result in a delay or reduction in colonization of germinating tree seedlings. The MPN counts of propagule density support this interpretation, and show the relative unimportance of spores as inoculum. Propagule density in sieved soil in undisturbed and selectively logged forest (*ca.* 300 propagules 100 g^{-1} soil) was at least ten times greater than spore density. The 'extra' propagules were undoubtedly root and hyphal fragments. In contrast, on the heavily logged site, propagule density was much lower (6–75 propagules 100 g^{-1} soil) but still up to ten times greater than spore density. The decline in propagules can be attributed to the reduction in infected living root length in the soil from the heavily logged site but also to the decline in infectivity over time of root fragments left in the soil after logging. The storage

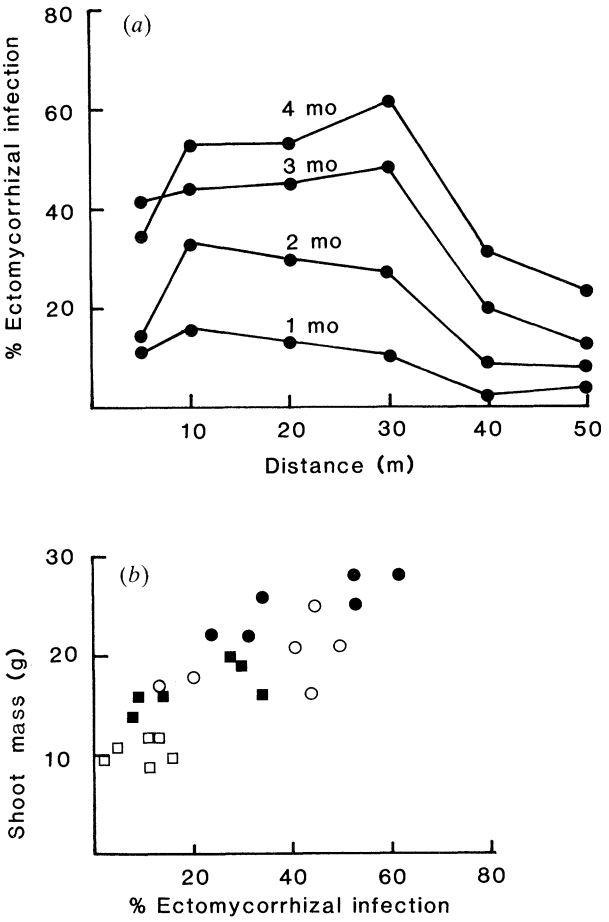

Figure 5. (*a*) Ectomycorrhizal infection (percentage root tips infected) of *Intsia palembanica* seedlings at increasing distance from a mature *Dipterocarpus costulata* assessed each month for 4 months ($n=4$). (*b*) Relation between shoot dry mass and ectomycorrhizal infection at the four sample times.

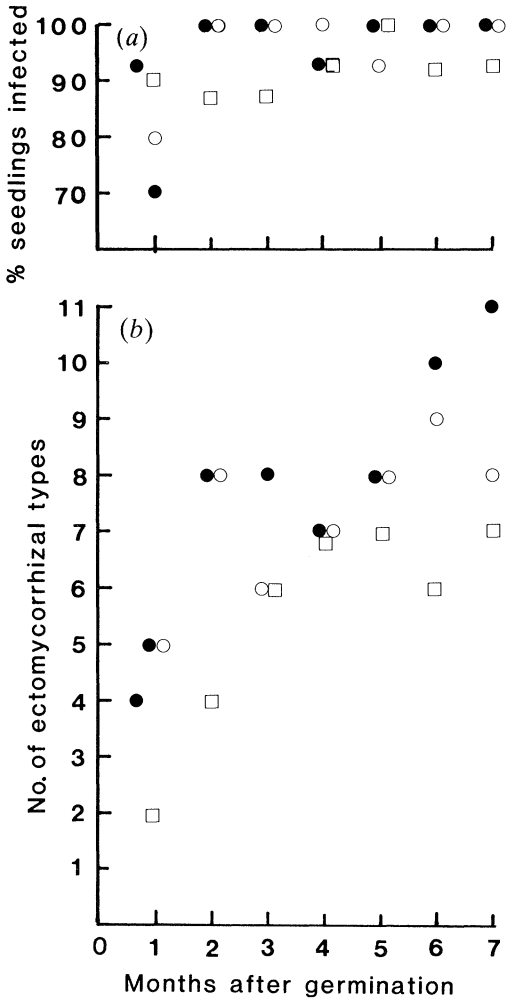

Figure 6. (*a*) The proportion of *Shorea leprosula* seedlings with ectomycorrhizas sampled 20 days to 7 months after germination at three secondary forest sites in Peninsular Malaysia ($n=10$–15). (*b*) The number of different ectomycorrhizal fungi associated with the seedling root systems at the three sites.

experiment suggested that an 80% reduction over 12 months was possible in soil devoid of vegetation.

That this reduction in propagule density does indeed result in reduced infectivity of soil was demonstrated by the reduced colonization of *A. falcataria* and *I. palembanica* grown in soil from the heavily logged site. Interestingly, colonization was also reduced in soil from the selectively logged site, although the propagule density there was the same as that on the undisturbed forest, and higher than the 100 propagules 100 g^{-1} estimated by Sanders & Sheik (1983) to saturate the infection process. This implies that changes in soil physical and chemical properties following logging (Ahmad 1989) may also adversely affect VA mycorrhizal infection.

Although the results of the bioassay of infectivity are in line with the measured reduction in spores, root infection and propagules following logging, the potted soil had in effect been doubly disturbed, once by the logging operation and then by sample collection and sieving. More precise information on how logging affects the onset and spread of infection could be obtained by germinating the test plants in the field where mycelial networks are intact.

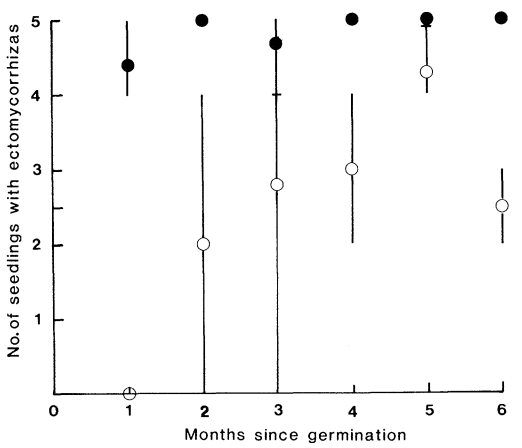

Figure 7. Ectomycorrhizal infection of *Shorea leprosula* seedlings in contact with (closed circles) or isolated from (open circles) the roots of parent trees. Each point represents the mean number of infected seedlings (out of a possible 5) at three sites in Semenyih Forest Reserve. Bars indicate the range of values observed.

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(*b*) *Growth response to mycorrhizal infection*

In some respects the results of the growth experiment were unexpected and very different from those normally reported in the literature (Harley & Smith 1983). Seedling growth responded more to mycorrhizal infection than to P addition and, although P addition reduced infection, at the highest rates the root systems were still quite heavily infected. This is despite the fact that available P (Bray 2 extraction) in the pots was increased from 3 $\mu g\ g^{-1}$ to 350 $\mu g\ g^{-1}$ by 1 g of TSP and to over 1500 $\mu g\ g^{-1}$ by 6 g TSP. Foliar analysis was only done on *Intsia palembanica* (Ahmad 1989). Shoot P concentration in uninfected plants rose from 400 $\mu g\ g^{-1}$ to 1000 $\mu g\ g^{-1}$ over the full range of P addition; in VA mycorrhizal plants shoot P concentration was consistently 40–50% higher. It is difficult to escape the conclusion that P availability was not the main factor limiting seedling growth under these particular experimental conditions, and that the control of mycorrhizal infection and the mycorrhiza-induced growth response were related to some factor or factors other than P supply. Despite the volume of literature on the effects of mycorrhizal infection, no comparable experiments on tropical woody species growing in tropical soils have been reported, and more work is required to confirm our findings.

The cocktail of 'introduced' VA fungi gave a much better growth response than the 'indigenous' inoculum, for comparable root length infected, particularly in the case of *Parkia bicolor*. The inoculation procedure was designed to minimize the risk of introducing pathogenic fungi from the forest, and the roots of the test seedlings showed no signs of pathogenic attack. It is tempting to see this result as support for Fitter's (1983) view that ineffective strains of VA mycorrhizal fungi are present in the field. However, some caution is required as the 'indigenous' fungi in the experiment may be strains selected out by the inoculation procedure and are not necessarily representative of the community existing in roots in the forest. Furthermore, the experiment was done in steam-sterilized soil. Nevertheless the difference between inocula, and the inoculum × host interaction, was striking. If, as seems likely, logging practice changes the composition of the community of VA mycorrhizal fungi as well as the overall inoculum potential this could be another factor influencing seedling regeneration.

Intsia palembanica seedlings with VA mycorrhizas grew better than those with ectomycorrhizas. Again this has to be interpreted with caution as the inoculation procedure will have selected for ectomycorrhizal fungi capable of infecting from chopped root fragments, and these may not be those which are important in the forest. The soil used in the pot experiments was low in organic matter and that may have influenced the outcome of the experiment. However, as the basis of the mycorrhizal response was not clear, an explanation for the better growth of the VA plants must await further experimentation.

(*c*) *Ectomycorrhizal infection of Intsia and Shorea*

The experiment at the Dipterocarp Arboretum provided circumstantial evidence that at least some ectomycorrhizal fungi infect both dipterocarps and *Intsia*. Two other points were made by the experiment. Firstly, seedling growth was correlated with percentage ectomycorrhizal infection. Of course, a number of factors other than mycorrhizal inoculum are likely to have been different at different distances from the arboretum, such as soil chemistry, soil temperature and the radiation climate, so no causal inference can be made. Smits (1983) suggested that the failure of dipterocarp seedlings planted in cleared areas was because high soil temperatures killed the mycorrhizal fungi. Bioassay experiments such as that described here could be useful to try to factor out mycorrhizal and other influences on dipterocarp regeneration in gaps of increasing size. The second point of interest was that the outplanted *Intsia* seedlings were ectomycorrhizal one month after germination, whereas those in pots were never infected at that stage. This shows the importance of contact with living roots for early ectomycorrhizal infection, a point which was confirmed by the two experiments with *Shorea*. In the first, seedlings growing in the forest were shown to be infected 20 days after germination, before they had true leaves. In the second, seedlings isolated from root contact remained uninfected in the field for up to 6 months. Read (1989) has stressed potential benefits to tree seedlings of incorporation into existing mycelial networks in terms of nutrient, water and carbon flow. Fleming *et al.* (1986) showed that *Betula* seedlings isolated from roots acquired different mycorrhizal fungi from those in contact with living roots. More work is required to assess the effects of different logging practice on the mycorrhizal status of dipterocarp seedlings, and to ascertain whether there are real benefits to seedlings of being in contact with living roots of parent trees.

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