SUCCESSFUL ECTOMYCORRHIZAL INOCULATION OF TWO DIPTEROCARP SPECIES WITH A LOCALLY ISOLATED FUNGUS IN PENINSULAR MALAYSIA

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LEE, S. S., PATAHAYAH, M., CHONG, W. S. & LAPEYRIE, F. 2008. Successful ectomycorrhizal inoculation of two dipterocarp species with a locally isolated fungus in Peninsular Malaysia. There is good potential for the application of ectomycorrhizas in improving dipterocarp seedling establishment, survival and growth through enhanced mineral nutrition and improved drought tolerance. However, these mycorrhizas must be easily propagated and applied as well as competitive with the native mycoflora, and indigenous ectomycorrhizal fungi are preferred. In this paper we report on the ability of a fungus isolated locally from ectomycorrhizas of *Shorea parvifolia* (Dipterocarpaceae) to form characteristic ectomycorrhizas with two different dipterocarp hosts, namely, *Hopea odorata* and *Shorea leprosula*. The fungus, designated FP160, has been provisionally identified as a member of the Thelephoraceae and efforts are on-going to identify it using molecular methods. FP160-inoculated *H. odorata* cuttings showed a positive growth response in the nursery but this effect was not significant when such cuttings were outplanted to a sandy tin tailing site. The smaller inoculated cuttings of *S. leprosula,* however, showed a significant growth response for up to three months in the field after outplanting but this effect disappeared thereafter. The synthesized FP160 ectomycorhizas on the dipterocarps were able to persist and remain dominant on inoculated plants for up to 23 months after outplanting in the sandy tin tailing site. FP160 appears to be a good candidate for enhancing the growth and development of plants in the nursery and in assisting the establishment of more fragile dipterocarps in the field.

Keywords: Growth response, rehabilitation of tin tailings, Thelephoraceae, tropics

LEE, S. S., PATAHAYAH, M., CHONG, W. S. & LAPEYRIE, F. 2008. Kejayaan inokulasi kulat ektomikoriza terhadap dua spesies dipterokarpa menggunakan kulat tempatan di Semenanjung Malaysia. Ektomikoriza mempunyai potensi yang baik dalam membantu penubuhan, kemandirian dan pertumbuhan anak benih dipterokarpa melalui pertambahan penyerapan nutrien dan peningkatan ketahanan kepada kemarau. Walau bagaimanapun ektomikoriza tersebut mestilah mudah dibiak dan digunakan serta dapat bersaing dengan mikoflora asal, dan kulat ektomikoriza asli adalah lebih disukai. Dalam kertas kerja ini kami melaporkan tentang kebolehupayaan sejenis kulat ektomikoriza tempatan yang berjaya dipencilkankan dari akar pokok *Shorea parvifolia* (Dipterocarpaceae) yang dapat membentuk ektomikoriza tipikal dengan dua perumah dipterokarpa yang berlainan, iaitu *Hopea odorata* dan *Shorea leprosula*. Kulat tersebut, yang pada masa ini dinamakan FP160, telah dikenal pasti daripada kumpulan Thelephoraceae dan usaha sedang dijalankan untuk mengesahkan identitinya melalui pendekatan kaedah molekular. Keratan pokok *H. odorata* yang diinokulasi dengan kulat FP160 di tapak semaian menunjukkan pertumbuhan yang lebih baik, namun ia tidak menunjukkan kesan yang signifikan apabila ditanam di lapangan di kawasan bekas lombong timah berpasir. Walau bagaimanapun keratan *S. leprosula* yang lebih kecil telah menunjukkan pertumbuhan yang lebih baik berbanding rawatan kawalan dalam tempoh tiga bulan pertama di lapangan, namun kesan tersebut hilang selepas tempoh tersebut. Ektomikoriza FP160 yang telah disintesis ke atas anak benih dipterokarpa didapati bertahan dan menjadi dominan pada anak benih yang telah diinokulasikan selama 23 bulan di lapangan di tanah lombong berpasir. FP160 mungkin menjadi calon yang baik untuk meningkatkan pertumbuhan dan perkembangan anak benih di tapak semaian dan membantu dalam pertumbuhan awal anak pokok dipterokarpa yang lebih mudah terjejas di lapangan.

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INTRODUCTION

In view of rapidly depleting areas of natural forests in South-East Asia, there is much interest in the establishment of plantations of both exotic and indigenous forest tree species, including members of the Dipterocarpaceae, to ensure future supplies of timber. The Dipterocarpaceae is the most important family of timber trees in Malaysia and South-East Asia and are among the most well-known trees in the tropics. Plantations of the fast growing exotics, *Acacia mangium* and *Eucalyptus* spp. have been extensively established in many South-East Asian countries while efforts to establish the much slower growing indigenous species such as the Dipterocarpaceae have lagged far behind for a variety of reasons (Appanah & Turnbull 1998), among which is the difficulty of ensuring successful survival and growth in the field.

As with other mycorrhizal symbioses, the dipterocarp ectomycorrhizal association has been demonstrated to enhance uptake of phosphorus and improve seedling growth in several dipterocarp species (Lee & Alexander 1994, Yazid e*t al.* 1994). Therefore, there is good potential for the application of mycorrhizas in improving dipterocarp seedling establishment, survival and growth through enhanced mineral nutrition and improved drought tolerance. The use of beneficial mycorrhizas may thus spur efforts to establish plantations of indigenous dipterocarps. However, these mycorrhizas must be easily propagated and applied as well as competitive with the native mycoflora, if they are to be readily utilized.

Conflicting results have been obtained in ectomycorrhizal synthesis experiments between dipterocarps and the exotic *Pisolithus arrhizus* (syn. *P. tinctorius* or Pt) in Malaysia. Successful synthesis of ectomycorrhizas between various strains of the fungus and several dipterocarp hosts has been demonstrated (Yazid *et al.* 1994, 1996, Lee *et al.* 1995) but ectomycorrhizal infection has been inconsistent. In some cases, levels of infection can be so extensive that the entire root system is colonized and yet in others, no infection is obtained with the same fungus strain and host (personal observation). Some dipterocarp species can form ectomycorrhizas when inoculated with Pt while others do not or only very few seedlings in the treatment become infected (Lee *et al.* 1995). These erratic results may be a consequence of poor adaptation of the exotic strains to either the dipterocarp host or to the Malaysian nursery environment. Another study from Malaysia demonstrated that two exotic strains of *Pisolithus* did not persist well on roots of three species of dipterocarp seedlings after outplanting (Chang *et al.* 1994). Their virtual disappearance from the well-infected and extensively colonized roots of inoculated plants six months after the plants had been outplanted in logged over forest suggests that they were probably not well adapted to or not competitive in the tropical rain forest environment. Results from some field experiments (Garbaye *et al.* 1988), and ultrastructural (Lei *et al.* 1990) and physiological studies of root infection (Albrecht *et al.* 1994) suggest that exotic strains of mycorrhizal fungi may be less aggressive in colonizing the host compared with indigenous strains which have co-evolved with the host tree.

In South-East Asia successful ectomycorrhizal syntheses have been reported between a number of indigenous ectomycorrhizal fungi and dipterocarps in Thailand (Sangwanit & Sangthian 1991) and Indonesia (Fakuara & Wilarso 1992, Ogawa 1992, Supriyanto *et al.* 1993). In Malaysia ectomycorrhizal synthesis experiments have been conducted with the indigenous ectomycorrhizal fungus, *P. aurantioscabrosus* (Watling *et al.* 1995) but unfortunately it did not form ectomycorrhizas with any of the four dipterocarp species tested (Lee *et al.* 1995).

Based on records and observations of basidiomata, the main groups of ectomycorrhizal fungi involved in the symbiotic association with dipterocarps are members of the Russulales, Amanitales, Boletales, Cantharellales and several hypogeous taxa; all members of the Basidiomycota (Watling & Lee 1995, Watling *et al.* 1998, Lee *et al.* 2002, 2003). Recent molecular data have, however, revealed that members of the Thelephoraceae are common ectomycorrhizal fungi associated with the Dipterocarpaceae and that they are most often associated with *Shorea* spp. (Sirikantaramas *et al.* 2003).

Therefore, there is still a need to find suitable indigenous ectomycorrhizal fungus inocula that can enhance the establishment, survival and growth of dipterocarps in the nursery and field, and which are also able to persist on the roots of the host plant and compete with naturally occurring mycorrhizas in the field. This paper reports on such a potential ectomycorrhizal fungus inoculum.

Three experiments were carried out. The first experiment was a preliminary ectomycorrhizal synthesis experiment conducted to test the ability of the candidate fungus to form ectomycorrhizas on roots of *Hopea odorata* seedlings. A second experiment was conducted to investigate the effect of a different form of inoculum of the same fungus on the growth of *H. odorata* cuttings. Following the success of the second experiment, a third experiment was conducted to examine the potential of using ectomycorrhizal inoculated *H. odorata* and *Shorea leprosula* cuttings for reforestation of a sandy tin tailing site (sandy wasteland left after tin mining). There are about 113 700 ha of heavily degraded sandy tin tailings in Peninsular Malaysia (2% of the total land area) needing immediate revegetation.

MATERIALS AND METHODS

Isolation of fungus

During a visit to the Forestry Department nursery at Lentang in the state of Pahang, Malaysia in August 2000, it was noted that seedling root systems of the dipterocarps, *Neobalanocarpus heimii*, *S. leprosula* and *Shorea parvifolia* were totally infected by a bright brownish ectomycorrhizal fungus. These seedlings were between nine and 12 months old and were planted in root trainers containing naturally composted oil palm mesocarp fibre (fibre and empty kernels remaining after extraction of oil from the mesocarp of the oil palm fruit) which had been exposed to the elements for six months. *Neobalanocarpus heimii* and *S. parvifolia* plants were taken back to the laboratory at the Forest Research Institute Malaysia (FRIM), Kepong for further examination and ectomycorrhizal roots of the latter were used for isolation of the associated fungus.

Shorea parvifolia roots were washed clean of soil in tap water and selected healthy, ectomycorrhizal root tips cut into 5 mm segments. Root segments were surface sterilized by soaking in a 1% sodium hypochlorite solution for 3 min before transferring into sterile distilled water. The root segments were rinsed 10 times with sterile distilled water under suction. Cleaned root segments were blotted dry with sterile filter paper before being placed on Pachlewski's agar (Pachlewski & Pachlewska 1974) in test tube slants, one segment per tube. The slants were placed in an incubator at 25 ± 2 °C and inspected daily to monitor development. Contaminated slants were destroyed.

One particular fungus was observed growing from root fragments in about 10% of the 200 tubes after about two weeks and was subcultured into Petri dishes containing Pachlewski's agar. Pure culture isolates of the fungus, designated isolate FP160, were kept on Pachlewski's agar slants and as plugs in sterile distilled water in an incubator at 25 ± 2 °C.

Peat:vermiculite inoculum preparation

A peat:vermiculite inoculum was prepared for large-scale inoculation. The inoculum substrate consisted of a peat:vermiculite mixture made up of a commercial tropical peat, marketed locally as Kosas-Peat (Kosas Profil Sdn. Bhd.) mixed with vermiculite in a ratio of 1:9 (v/v). A total of 150 g l^{-1} peat was thoroughly mixed into Pachlewski's liquid media using a magnetic stirrer for two hours before adjusting the pH to 5.5. Then 200 ml of the peat-Pachlewski's mixture was added to 270 ml (v) of sieved vermiculite contained in 15 × 23 cm high density polyethylene bags fitted with a 4 cm diameter PVC ring (forming a neck). The opening at the neck of the bag was then covered with a double layer of muslin cloth, fastened with a wire ring and capped with aluminum foil before being autoclaved at 121 °C for 20 min.

Eight plugs of FP160 growing on Pachlewski's agar were cut from the actively growing edge of 2-week-old colonies with a 1 cm diameter sterile cork borer and transferred onto the cooled media in each plastic bag under sterile conditions. The inoculated bags were kept in the dark in an incubator at 25 ± 2 °C until the substrate was well colonized by the fungal mycelium. This took about one and a half months. Inocula were leached under running tap water to remove excess sugars before use.

Ectomycorrhizal synthesis experiments

Experiment 1: Seeds of *H. odorata* (family: Dipterocarpaceae) collected from parent trees growing on the grounds of FRIM were germinated in washed river sand prior to transplanting. Eighteen 1-month-old seedlings

were transplanted into root observation boxes (30 × 15 × 1 cm) (Lee 1991) filled with steam sterilized (steaming at 90 °C for 1 hour for two consecutive days), well-composted oil palm mesocarp fibre obtained from the Forest Department nursery in Lentang. Seedlings were placed on the surface of one side of the planting medium such that the roots were visible through one side of the root observation box. At transplanting, FP160 inoculum prepared on cardboard (6 × 3 cm) (Chilvers *et al.* 1986) was laid over the visible seedling roots before the side of the root observation box was replaced. Nine seedlings were inoculated while nine uninoculated seedlings acted as controls. The root observation boxes were wrapped in black plastic sheets and the plants kept under shade at the FRIM nursery. The cardboard inocula were removed after two weeks. Roots of all plants were inspected under the stereomicroscope three days later and every other day thereafter to monitor ectomycorrhizal development.

Experiment 2: One hundred cuttings of *H. odorata* were prepared according to Aminah *et al.* (2001). During potting one heaped teaspoonful of the leached peat:vermiculite inoculum was placed in contact with roots of each cutting in a black polyethylene planting bag containing composted oil palm mesocarp fibre (Aminah *et al.* 2003). Roots of the cuttings were non-mycorrhizal at the time of inoculation. One heaped teaspoonful of the peat:vermiculite inoculum prepared as described above but not inoculated with the test fungus was used for the uninoculated control cuttings. There were 50 cuttings in each treatment. All cuttings were then transferred to the FRIM shade house and watered carefully once daily.

The height of the cuttings from soil level to the tip of the tallest shoot was measured at monthly intervals and the experiment was terminated after six months. At the end of the experiment 40 plants from each treatment were randomly sampled for total root length and total biomass while the remaining 10 were used for determination of percentage ectomycorrhizal infection. Plants sampled for total root length and biomass were dried in an oven (ULM 500 Memmert, Germany) at 50 °C over 72 hours until constant weight was obtained.

Data was analyzed using the SPSS (Version 12) program. The means of the height, total root length and biomass of both inoculated and uninoculated plants were compared using independent samples *t*-test. The results of the analysis were considered significant when the probability level was equal to or less than 5% ($p \leq 0.05$).

Experiment 3: A total of 200 cuttings of the dipterocarps *H. odorata* and *S. leprosula* were prepared and inoculated as described in Experiment 2 above. One hundred cuttings of each species were inoculated with the ectomycorrhizal peat:vermiculite inoculum while the remaining cuttings were used as uninoculated controls. All cuttings were then transferred to the FRIM shade house and watered carefully once daily.

Three plants of each species were randomly sampled at weekly intervals for up to one month to monitor ectomycorrhizal infection. Thereafter, three plants per species were randomly sampled once a month for another five months to monitor ectomycorrhizal development. In the sixth month, all plants were transplanted to a tin tailing site in Bidor, Perak in an outplanting experiment as shown in Figure 1. In each plot the cuttings were planted in between the existing 3-year-old *A. mangium* nurse trees as young dipterocarp plants usually require overhead shade for survival and good growth (Weinland 1998); 20 cuttings of *H. odorata* in each third and fourth row and 20 cuttings of *S. leprosula* in each seventh and eighth row. Inoculated plants were planted in the plots designated +M while uninoculated control plants were planted in the plots designated –M. Growth of plants measured as root collar diameter was recorded at one, three, seven and 11 months after planting. Growth measurements which had been scheduled for the sixth and twelfth months had to be made at seven and 11 months respectively due to unavoidable logistical problems. Growth measurements were discontinued after 11 months as the site became heavily disturbed and some trees were damaged due to incursions by wandering, grazing cattle.

At three months after planting roots of both inoculated and uninoculated *H. odorata* and *S. leprosula* plants were sampled using a 65 mm diameter soil corer and examined

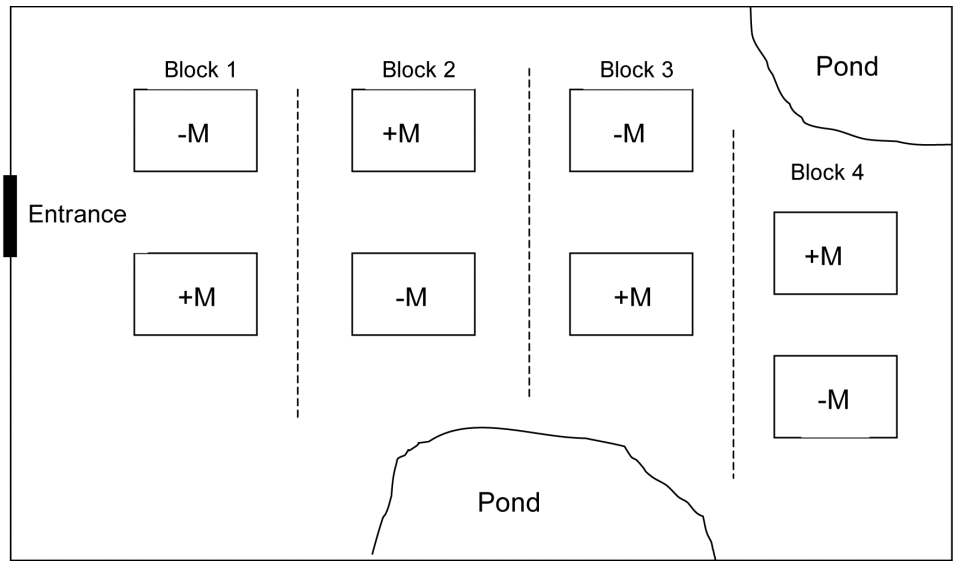

Figure 1 Plot layout for the outplanting experiment on a sandy tin tailing site in Bidor, Perak with four blocks, each consisting of two plots. Each plot was planted with 10 rows of 20 one-year-old *Acacia mangium* seedlings in March 2000. In March/April 2002, 20 rooted cuttings per row of inoculated *Hopea odorata* and *Shorea leprosula* were planted in between the *A. mangium* in rows 3 and 4, and rows 7 and 8 of plots designated +M and the same number of uninoculated, control *H. odorata* and *S. leprosula* rooted cuttings planted in between the *A. mangium* in the same rows in the plots designated –M.

for ectomycorrhizal infection. Roots from six inoculated and three uninoculated *H. odorata* and two each inoculated and uninoculated *S. leprosula* plants were randomly sampled. Fewer *S. leprosula* plants were sampled because of their limited availability due to the high mortality rate; 52% of inoculated plants and 45% of uninoculated plants had died after two months compared with only 1% mortality in *H. odorata*. After 23 months of outplanting (i.e. 29 months after inoculation) roots of inoculated and uninoculated plants were again sampled using a soil corer for examination of mycorrhizas. Roots of 20 plants each of inoculated and uninoculated *H. odorata* and nine inoculated and 11 uninoculated *S. leprosula* were sampled.

RESULTS AND DISCUSSION

The mantle features and hyphal characteristics of FP160 generally resembled those of *Tomentella* (Agerer *et al.* 2001) or *Thelephora* ectomycorrhizas (Agerer 1987–1995, Ingleby *et al.* 1990) but there are some differences. As the fungus does not produce any visible basidiomata in culture, efforts are currently on-going to identify it using molecular methods and the results will be published in a separate paper. In the meantime, it has been provisionally identified as a member of the Thelephoraceae. Recent molecular data has revealed that members of the Thelephoraceae are common ectomycorrhizal fungi associated with the Dipterocarpaceae in Malaysian forest (Sirikantaramas *et al.* 2003). It is highly likely that this fungus is an indigenous species as it was isolated from roots of a *S. parvifolia* seedling grown on locally obtained substrates in the Lentang nursery which is located in a valley surrounded by selectively logged and unlogged dipterocarp forests.

In Experiment 1, earliest development of FP160 ectomycorrhizas was observed six days after removal of the cardboard inoculum on one *H. odorata* seedling in the root observation boxes. By the third week, eight of the nine inoculated plants had developed extensive ectomycorrhizas and the last plant became infected by the fourth week. Infection was not only confined to the feeder roots but also present on the taproot where a well-developed mantle and Hartig net were formed. The extensive colonization of the taproot by the ectomycorrhizal fungus was probably a consequence of forced mycorrhization under the experimental conditions as has been demonstrated elsewhere (Dexheimer *et al.* 1994). Roots of the uninoculated control seedlings were

still non-mycorrhizal at the end of the first week but after three weeks about half of the uninoculated control seedlings had become infected with contaminant ectomycorrhizas that probably originated from the nursery. After two months only one control seedling remained uninfected. The contaminant ectomycorrhizas were of two types commonly found on dipterocarps at the FRIM nursery. One type, a whitish to light brown ectomycorrhiza with a thin mantle, is most likely that formed by *Inocybe* sp., while the other, which is dark brown and designated as T2, is of an unknown basidiomycete. Fruiting bodies of *Inocybe aurantiocystidiata* Turnbull & Watling (Turnbull 1995) are frequently encountered at the FRIM nursery (personal observation) and could have been one of the sources of the contaminant ectomycorrhizas. When the experiment was terminated after two and a half months, FP160 ectomycorrhizas were still extensively present on roots of inoculated plants with no signs of infection by other contaminant ectomycorrhizas in contrast to control seedlings which had become contaminated with at least two different nursery ectomycorrhizas. This suggests that FP160 could be a strong competitor under nursery conditions. On the taproot, however, FP160 ectomycorrhizas were no longer present. The mycorrhizas were now mainly confined to the top half of the root system while newly developed roots in the lower half of the root system were uninfected or had yet to become infected. This was probably due to depletion of nutrient resources in the confined space of the boxes since these seedlings were not supplied with any additional nutrients during the experimental period.

The results from Experiment 2 showed that *H. odorata* cuttings inoculated with FP160 had significantly better shoot growth than uninoculated controls from three months onwards (Figure 2). At the end of six months ectomycorrhizal inoculated plants were 30% taller, had 62% longer total root length and 40% higher biomass than uninoculated control plants (Table 1a). Of the 10 inoculated plants whose roots were inspected for ectomycorrhizal infection at the end of the experiment, all were colonized by FP160 with an average infection rate of about 53%. Three of these plants also had the *Inocybe*-like ectomycorrhizas observed in Experiment 1 but infection levels were low (3%). One uninoculated plant had about 1% infection by FP160 ectomycorrhizas while seven other plants had about 30% infection by a combination of the *Inocybe*-like ectomycorrhizas, the dark brown type designated T2 (see above) and *Cenococcum*-like ectomycorrhizas (Table 1b). The FP160 mycorrhizas which were found on the uninoculated plant could have spread from the neighbouring FP160 inoculated plants while the contaminant ectomycorrhizas probably came from the nursery. These results showed that while FP160 ectomycorrhizas could be relatively easily synthesized, they also resulted in improved

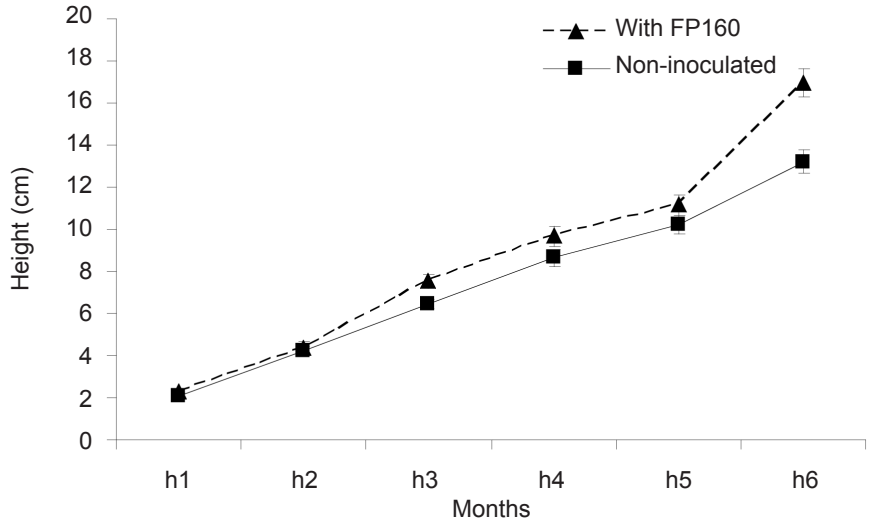

Figure 2 Monthly mean (± SE) shoot height of *H. odorata* cuttings grown on composted mesocarp fibre after six months at the FRIM nursery in Kepong

Table 1(a) Mean (± SE) shoot height, total root length and total biomass (dry weight) of *Hopea odorata* cuttings grown on composted mesocarp fibre after six months at the FRIM nursery in Kepong

Growth parameter	Inoculated with FP160	Uninoculated
Shoot height (cm)	17.1* ± 0.68	13.1 ± 0.55
Total root length (cm)	146.8* ± 10.25	90.7 ± 10.76
Biomass (g)	1.88* ± 0.10	1.35 ± 0.10

* indicates significant difference at $p < 0.05$

Table 1(b) Percentage infection of FP160 inoculated and uninoculated *H. odorata* cuttings after six months at the FRIM nursery in Kepong

Treatment	FP160 ectomycorrhizas (%)	Contaminant ectomycorrhizas (%)	Non-mycorrhizal tips (%)	Dead root tips (%)
Inoculated plants	52.9	3.0	40.1	4.0
Uninoculated plants	1.1	30.4	65.0	3.5

growth of *H. odorata* plants. Thus we decided to test the benefit of inoculating dipterocarps with FP160 for outplanting in a sandy tin tailing site in Experiment 3.

In Experiment 3, cuttings of both *H. odorata* and *S. leprosula* developed characteristic FP160 ectomycorrhizas seven days after inoculation. Weekly inspections revealed successful infection and progressive development of FP160 ectomycorrhizas on roots of both dipterocarp species. At outplanting six months later, FP160 mycorrhizas were still abundant on roots of inoculated plants of both species. Random root inspection of uninoculated plants revealed that roots of some plants had low levels (about 10%) of infection by contaminant ectomycorrhizas similar to those observed in Experiment 2. Three months after outplanting, examination of roots of randomly selected *H. odorata* and *S. leprosula* plants showed that FP160 ectomycorrhizas were still extensively present on all the inoculated plants although some contaminant mycorrhizas were also present.

The high mortality of *S. leprosula* plants in the field could have been due to the use of insufficiently mature plants for outplanting. Normally, dipterocarps are planted as potted seedlings when they are about nine months old and about 25–30 cm tall (Weinland 1998). However, because of time constraints, six-month-old plants had to be used and due to the relatively poor success rate of raising sufficient numbers of *S. leprosula* cuttings in the scheduled time, a high proportion of cuttings were less than 15 cm tall when outplanted. In contrast, the *H. odorata* cuttings were all more than 15 cm tall at the time of outplanting. The species is also known to be able to grow well on degraded sites (Wan Razali & Ang 1991).

There was no significant difference in root collar diameter increment of inoculated and uninoculated *H. odorata* plants at one, three, seven and 11 months after outplanting. In contrast, inoculated *S. leprosula* plants had significantly higher growth rates up to three months after planting but this effect disappeared by the seventh month (Table 2). Thus, although the ectomycorrhizal inoculum FP160 had a positive effect on growth of *H. odorata* for up to six months in the nursery, the mycorrhizal effect was not evident once the plants had become established in the field even though FP160 was still present on the roots. However, with the smaller perhaps more fragile and probably less well adapted *S. leprosula*, FP160 continued to enhance the growth of the cuttings up to three months more in the field. The absence of the ectomycorrhizal effect in the field could have been a result of differences in the rate of spread of the inoculum on the root system or because of possible antagonistic effects due to grasses and weeds at the site or other soil microorganisms (Le Tacon *et al.* 1992). It has been reported that in poor sites without sufficient P application,

Table 2 Mean (± SE) root collar diameter of *H. odorata* and *Shorea leprosula* cuttings at one, three, seven and 11 months after outplanting in a sandy tin tailing site under *A. mangium* nurse trees in Bidor, Perak

Month	Mean root collar diameter (cm)			
	H. odorata		*S. leprosula*	
	Uninoculated	Inoculated with FP160	Uninoculated	Inoculated with FP160
1	3.66 ± 0.09	3.34 ± 0.13	2.47 ± 0.09	3.54 ± 1.96*
3	7.97 ± 0.15	7.06 ± 1.45	2.69 ± 0.17	4.07 ± 0.25*
7	10.05 ± 0.33	10.76 ± 0.41	5.34 ± 0.59	4.75 ± 0.50
11	16.27 ± 1.07	17.22 ± 0.60	11.68 ± 1.43	7.85 ± 1.44

* indicates significant difference at $p < 0.05$

inoculation can sometimes have a depressive growth effect (Le Tacon *et al.* 1992). Other studies have shown that while strong host tree growth promotion can be obtained in sterile conditions, such a positive response is absent in non-sterile conditions in the field (Jumpponen & Trappe 1998). Since FP160 was originally isolated from nursery plants, it could be that it is a member of the so-called nursery fungi which are usually not dominant members in forest mycorrhizal communities or in this case at the sandy tailing site which suggests that it may have a poor competitive capacity after transplanting (Heinonsalo 2004). There is also the possibility that the naturally occurring ectomycorrhizal fungi at the site were sufficiently adapted to the dipterocarp plants to have a competitive superiority that reduced effectiveness of the introduced strains (Le Tacon *et al.* 1992).

After 23 months in the field, i.e. 29 months after inoculation, FP160 ectomycorrhizas were found to be still persistent (Table 3a) and dominant, infecting more than 60% of the root tips (Table 3b) of both inoculated *H. odorata* and *S. leprosula* plants. Some contaminant ectomycorrhizas were found on 40% of the inoculated *H. odorata* and 50% of the *S. leprosula* plants but infection was low, with less than 15% of the root tips infected (Table 3b). These contaminant mycorrhizas originated from the field as they were found to be morphologically different from the nursery contaminant types observed in Experiments 1 and 2. Although a contaminant ectomycorrhiza was found on 75% of root tips of one inoculated *S. leprosula*, it appeared that FP160 was able to persist and remain dominant on roots of the inoculated plants for nearly two years after plantation, unlike some fungi which disappear within a few weeks (Chang *et al.* 1994, Lee *et al.* 1995, Frey-Klett *et al.* 1997). This is not unexpected as introduced strains of ectomycorrhizal fungi have been found years after inoculation (Stenström 1990, Le Tacon *et al.*, 1992, Selosse *et al.* 1998).

Le Tacon *et al.* (1992) clearly showed that the growth response of inoculated plants in the field can be quite variable and that the vigour of the natural ectomycorrhizal inoculum is the most important factor in determining the success or failure of the inoculation. Moreover, the introduced fungi must be adapted to the ecological conditions of the reforestation site. It is therefore recommended that the search continue for suitable strains of ectomycorrhizal fungi and that they be tested under non-sterile conditions using natural soil or soil from the test field site.

CONCLUSIONS

The ectomycorrhizal fungus FP160 can be easily cultured on artificial media and grows fairly rapidly. It is able to successfully form ectomycorrhizas with different dipterocarp hosts, namely, *H. odorata* and *S. leprosula*. On inoculated plants ectomycorrhizal formation occurred after about a week and a positive growth response was observed on *H. odorata* seedlings in the nursery. Therefore, FP160 shows good potential as an ectomycorrhizal inoculum for enhanced growth of dipterocarp seedlings and cuttings in the nursery. Inoculation with FP160 had a positive growth effect on the smaller *S. leprosula* cuttings up to three months in the field after outplanting, but there was no significant

Table 3(a) Persistence of ectomycorrhizal infection by FP160 on *H. odorata* and *S. leprosula* plants 23 months after outplanting (29 months after inoculation) in a sandy tin-tailing site in Bidor, Perak

Host species	No. plants sampled	No. plants with FP160 mycorrhizas	No. plants with contaminant mycorrhizas
H. odorata (inoculated)	20	20	8
H. odorata (uninoculated)	20	0	18
S. leprosula (inoculated)	9	8	4
S. leprosula (uninoculated)	11	0	8

Table 3(b) Mean percentage infection by FP160 on *H. odorata* and *S. leprosula* plants 23 months after outplanting (29 months after inoculation) in a sandy tin tailing site in Bidor, Perak

Host species	% infection by FP160	% contaminant infection	% non-mycorrhizal tips
H. odorata (inoculated)	60.8	13.8	19.2
S. leprosula (inoculated)	60.5	8.1	20.3
H. odorata (uninoculated)	0	77.3	11.2
S. leprosula (uninoculated)	0	66.2	16.4

growth response on *H. odorata* cuttings even at one month after outplanting. The fungus was, however, still dominant on inoculated *H. odorata* and *S. leprosula* even after the plants had been planted out in a tin tailing site for 23 months. Research is on-going to identify the fungus using molecular techniques. The ease of growing this fungus on artificial media, its rapid growth and easy manipulation and persistence, and its growth benefits for the host plant in the nursery make it a good candidate for a dipterocarp ectomycorrhizal inoculation programme in the nursery. However, since FP160 does not appear to enhance plant growth in sandy tin tailings, further research needs to be carried out to search for other suitable fungi which are able to enhance plant growth both in the nursery and in such degraded sites. In addition, further studies should also be conducted with FP160 as it is still unclear how and whether all species respond in the same way to mycorrhizal infection in various ecosystems.

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