PROCEEDINGS OF THE 6TH INTERNATIONAL WORKSHOP OF BIO-REFOR, BRISBANE, AUSTRALIA

DECEMBER 2–5, 1997

Overcoming Impediments to Reforestation: Tropical Forest Rehabilitation in the Asia-Pacific Region

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Published by BIO-REFOR

ISBN4-90661-77-7 C3061 P3900E

March 1998

Poster Papers

Field Performance of *Eucalyptus urophylla* Inoculated with an Introduced and Indigenous Strains of *Pisolithus* at Three Sites in the Philippines

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ABSTRACT

The effectiveness of an isolate of *Pisolithus* from Australia was compared with a Philippine *Pisolithus* isolate in promoting the growth of *Eucalyptus urophylla* on three acid (pH 4.1–5.9, 0.005 *M* $CaCl_2$) sites in the Philippines (Pangasinan, Bukidnon and Surigao). Isolates of *Pisolithus* were taken from basidiocarps collected under eucalypts growing in Western Australia and from the Philippines. Generally, the introduced *Pisolithus* promoted greater wood volume of *E. urophylla* planted in dry marginal land (Pangasinan) and in moist logged-over area (Surigao) in the Philippines than the Philippine *Pisolithus* isolate. Root colonization by the two fungi did not vary but there was a difference in the root colonization levels between sites implying that the prevailing microclimatic conditions on each site had affected the performance of the ECM inoculants. In this study, the number of isolates tested was limited, thus, future field trials should include a wider range of ectomycorrhizal fungi. Further work is required to determine whether the growth responses measured at the two sites (Pangasinan and Surigao) are maintained until the trees are harvested.

Key words: ectomycorrhizal fungi, *Pisolithus*, *Eucalyptus urophylla*

INTRODUCTION

Eucalyptus urophylla S.T. Blake is one of the best low-latitude eucalypts for planting for timber in the tropics. Natural stands of this eucalypt species are common in Indonesia (Eldridge *et al.*, 1993). When introduced to other countries, it is often superior to other eucalypt species in producing wood, grows well in dry sites and on strongly acidic soil (Simoes *et al.*, 1980) and tolerant of heavy metals (Neves *et al.*, 1982). The adaptation of this eucalypt species in such inhospitable environments may depend on the presence of symbiotic mycorrhizal associations. Ectomycorrhizal (ECM) symbioses can increase tree growth in acid soils where the availability of essential nutrients, particularly P, are low (Mengel & Kirkby, 1987). Eucalypts are naturally associated with ectomycorrhizal fungi (Chilvers & Pryor, 1965) and are strongly dependent on mycorrhizal symbionts for growth in soils of low nutritional status (Malajczuk *et al.*, 1975). *Pisolithus*, an ectomycorrhizal fungus has the potential to improve growth and survival of trees planted on sites with adverse conditions such as nutrient deficient and heavy metal contaminated soils. Furthermore, *Pisolithus* isolates can grow well and form mycorrhizas at relatively high temperatures (Jeffries & Dodd, 1991).

Field trials on *Pisolithus*-eucalypt have been reported by Delwaulle *et al.* (1987) and Garbaye *et al.* (1988) in the Congo, Malajczuk *et al.* (1994) in China, Grove *et al.* (1991) in Western Australia and De la Cruz *et al.* (1990) in the Philippines. Initial inoculation trials of *E. camaldulensis* and *E. deglupta* in the Philippines have used a mixture of spores of *Scleroderma* sp. and *Pisolithus* sp. collected under established pine plantations (De la Cruz *et al.*, 1990). Significant height and diameter growth increases were obtained initially in the nursery and significant growth gains were obtained in the field even after 2 years (De la Cruz *et al.*, 1990). At this stage, there is only one isolate of *Pisolithus* that has been observed under eucalypt plantations in the Philippines. It is therefore interesting to compare the effectiveness of *Pisolithus* isolates collected under eucalypt stands in Western Australia and in the Philippines in promoting tree growth under field conditions. In a glasshouse trial, Aggangan *et al.* (1996a) reported that some Australian *Pisolithus* stimulated growth of *E. urophylla* in a non-sterile, acid Australian soil. However, the same authors (Aggangan *et al.*, 1996b) found that the Australian *Pisolithus* isolate was markedly affected by biological factors in unfumigated soil.

This study reports field trials set up to determine the growth response of *E. urophylla* seedlings inoculated with an Australian and a Philippine isolate of *Pisolithus* at three acidic sites in the Philippines.

MATERIALS AND METHODS

Biological materials

Fruit bodies of the ECM fungus *Pisolithus* were collected under eucalypts growing in Western Australia (A) and in the Philippines (P). Aseptically germinated seedlings were overlaid on fungal mat and left for 3 weeks for root colonization. Uninoculated seedlings were grown without the fungus. Mycorrhizal and non-mycorrhizal seedlings were planted individually in WG7 tubes filled with autoclaved 2:1 (v/v) peat perlite mixture. Seedlings for outplanting in Pangasinan were raised at BIOTECH, University of the Philippines at Los Banos while seedlings for Bukidnon and

Surigao were raised in their respective, on-site nurseries. The seedlings were grown for 4 months in the nursery under shade and eventually exposed to direct sunlight two weeks prior to outplanting. The seedlings were watered once a week (about 1 ml per seedling) with dilute complete fertilizer (3ml Wuxal foliar fertilizer L^{-1} H_2O) starting at 2 months.

Field sites

The experiment was established in three sites in the Philippines: Pangasinan, Luzon; Bukidnon, Mindanao and Surigao, Mindanao. All sites were acidic and low in available P (Aggangan *et al.*, 1996b). Field sites in Pangasinan and Bukidnon were former monsoon forests and now grassland whereas the field site in Surigao was a recently logged-over area. Pangasinan has climatic type 1, with two pronounced dry seasons (November to May) and wet season (June to October); Bukidnon has climatic type 3, with distinct short dry season (December to April) and long wet season (May to November); Surigao has climatic type 4, with no distinct dry season with a very pronounced maximum rainfall from November to March. Rainfall in the sites ranges from 1,000 mm (Pangasinan) to 3,500 mm (Surigao). Site preparations were either burning the vegetation in the area (Pangasinan), mechanically ripped (Bukidnon) or manually slashed (Surigao).

Experimental design

The experiment was established following a Randomized Complete Block Design with four blocks. Each treatment consisted of 11 trees per row plot planted in a hole (30 cm wide and 40 cm depth) with a spacing of 1 m and spacing between rows was 2 m in Pangasinan and Bukidnon and 4 m in Surigao.

Application of basal fertilizer

Basal fertilizers were applied as follows: in Pangasinan, 100 g urea at planting and another 100 g one-month after planting, 75 g of 14:14:14 (NPK) plus 50g micronutrient fertilizer mix (in %: 1.34 Cu, 0.22 Zn, 0.13 Co, 2.52 Mn, 5.56 Mg, 5.13 Fe, 0.20 Mo and 0.11 B), in Bukidnon, 160 g urea and 130 g NPK plus 25 g micronutrients, in Surigao only 100 g of NPK was applied 3 months after outplanting. Fertilizers were either mixed with the back fill soil in the planting hole (Pangasinan) or broadcasted in a circle on the soil surface approximately 25 cm from the base of the seedlings (Bukidnon and Surigao).

Parameters measured

Mycorrhizal infection was assessed before and after outplanting after clearing and staining the roots with tryphan blue. Height and diameter at 10 cm above the ground was measured periodically. Wood volume was calculated.

Statistical analyses

All data were analyzed in a one-way analysis of variance employing the General Linear Model of the Minitab Stat Program due to unequal number of trees per plot brought about by death of trees.

RESULTS

Mycorrhizal infection

Ectomycorrhizal infection on all seedlings for the three sites before outplanting were less than 10%. After 2 years, percentages of root tips colonized by the Australian and the Philippine *Pisolithus* were 30% and 36%, respectively, in trees planted in Pangasinan, 15% and 5% in Bukidnon and 5% and 2% in Surigao (Table 1). Cross sections of root infected with the Australian isolate revealed a thin mantle, well defined Hartig net and elongated epidermal cells. By contrast, the roots infected with the Philippine isolate had a thick and loose fungal mantle. After 2 years, the uninoculated trees planted in Pangasinan became infected with *Pisolithus* whereas the uninoculated trees planted in Bukidnon became infected with an unidentified indigenous *Scleroderma* spp. (3%), which however did not infect the inoculated trees. On the other hand, the uninoculated trees in Surigao had no mycorrhizas, 22 months after outplanting.

Table 1. Percentage of fine roots of inoculated and uninoculated *E. urophylla* trees colonized by ECM and VA fungi, 26 months after outplanting in three field sites in the Philippines. Values are means of 8 observations with standard deviation.

Site/ Inoculation treatment	ECM infection (%)	VA infection (%)	Remarks
Pangasinan			
Uninoc	12±11	0	—
A	30±6	0	few *Glomus* spp. spores attached to roots
P	36±3	3±2	vesicles present
Bukidnon			
Uninoc	3±3	2±3	vesicles and spores of *Glomus* spp. present
A	15±4	5±2	vesicles and spores of *Glomus* spp. present
P	5±2	2±1	vesicles and spores of *Glomus* spp. present
Surigao			
Uninoc	0	0	spores of *Acaulospora* sp. present on roots
A	5±3	0	numerous loose hyphae around the roots, spores of *Acaulospora* spp. present on roots
P	2±2	0	spores of *Acaulospora* spp. present on roots

Seedling mortality

In all sites, the highest mortality was observed in the uninoculated treatments: 18% in Pangasinan and in Bukidnon at 26 months, and 47% in Surigao at 22 months after outplanting. The lowest mortality in Pangasinan was observed in trees inoculated with the Australian isolate (9%), whereas in Bukidnon, the lowest was observed in trees inoculated with the Philippine isolate (9%).

Plant growth

The Australian isolate promoted better tree growth than the Philippine isolate. In Pangasinan, the Australian *Pisolithus* increased wood volume by 37% and in Bukidnon by 69% at 26 months compared with the uninoculated controls (Fig. 1). In Bukidnon, however, the two isolates of *Pisolithus* pro-

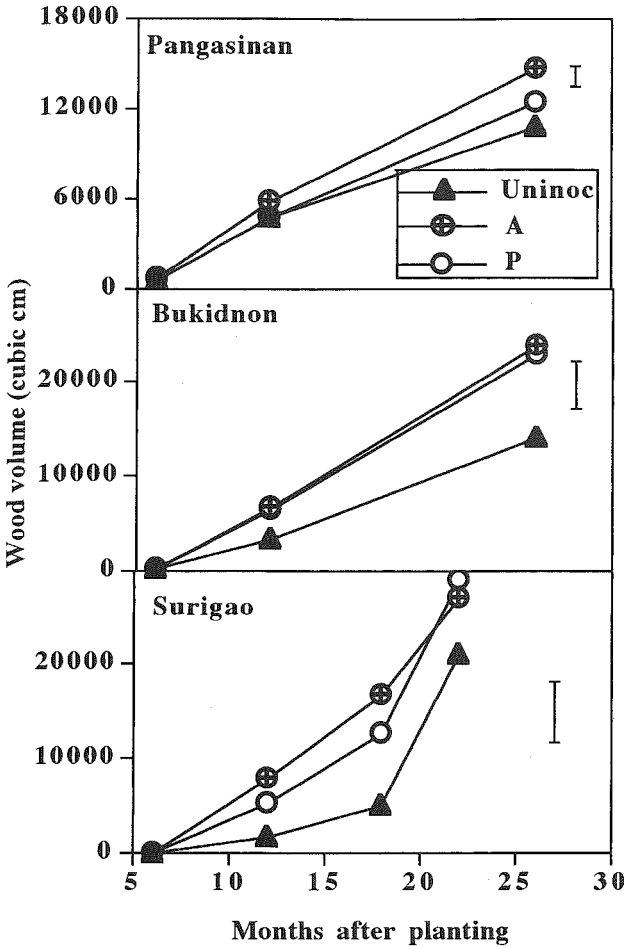

Fig. 1. Wood volume of *E. urophylla* seedlings inoculated with isolates of *Pisolithus* from Australia (A) and from the Philippines. Bars represent LSD value at $p<0.05$ at 26 months (Pangasinan and Bukidnon) or 18 months (Surigao) after outplanting.

moted similar wood volume. In Surigao, the Australian isolate increased wood volume by 237% at 18 months after outplanting. The Philippine isolate also increased wood volume but to a lesser extent (16% in Pangasinan, 63% in Bukidnon and 153% in Surigao).

DISCUSSION

Effectiveness of *Pisolithus* isolates in promoting plant growth

Inoculation with *Pisolithus* promoted the early growth of *E. urophylla* trees on acid soils at three sites in the Philippines. The Australian isolate promoted larger wood volume in Pangasinan over the two years growth period and during early growth up to 18 months in Surigao. At Bukidnon, however, both isolates of *Pisolithus* promoted similar wood volume during two years growth in the field. This implies that the growth promoting ability of *Pisolithus* is site dependent. Differences may be attributed to the microclimatic conditions in the experimental sites and to soil properties such as microbial populations.

Similar field trials have been established on acidic and infertile sites in Southern China to compare Australian and Chinese ectomycorrhizal fungi in promoting the growth of eucalypts. In Yunnan Province, the Australian *Pisolithus* (H445, similar to that used in this study) and the Chinese *Pisolithus* isolates promoted similar basal area of *E. globulus* during the three years growth period at three sites (Dagan *et al.*, 1995). Likewise, field trials in Australia have shown early growth promoting abilities by some ectomycorrhizal fungi (Thomson *et al.*, 1994). Largest increases in growth of eucalypts from inoculation with ectomycorrhizal fungi occur during the first 2 years after outplanting (Malajczuk *et al.*, 1994) and it is the stage when the nutrient supply from soil is most likely to limit tree growth (Grove & Malajczuk, 1994).

Persistence of *Pisolithus*

The two isolates of *Pisolithus* apparently survived in the roots of *E. urophylla* trees even up to 26 months after planting in all three sites in the Philippines. The infected roots were distinctly yellow in colour and had numerous, yellow, extramatrical hyphae similar to those observed in previous glasshouse experiments (Aggangan *et al.*, 1996a, b).

At 26 months, infection levels in Pangasinan were larger than in the other two sites but no fruit bodies of *Pisolithus* were observed in the area. The larger levels of infection in Pangasinan may be accounted for by the lack of competition from other ectomycorrhizal fungi or simply because the soil microflora fostered root colonization by the inoculant fungi since the initial level of infection prior to outplanting was low (less than 10%). By contrast, root infection levels in the inoculated seedlings planted in Bukidnon and more strikingly, in Surigao, were very low. The roots showed no colonization by any native ectomycorrhizal fungi such as a *Scleroderma* sp. which was prevalent (in September 1993) in Bukidnon.

In Bukidnon, even though the level of infection at 26 months after outplanting was low, it greatly increased wood volume. This implies that although there were few colonized roots, there might be numerous external hyphae. A positive effect of inoculation has also been reported on highly disturbed sites (as in the logged-over site in Surigao) with low inoculum potential (Grove & Le Tacon, 1993). Survival of introduced ectomycorrhizal fungi for long periods of time (greater than 2 years) does not appear to be a requisite for plant growth response to ectomycorrhizal inoculation (Garbaye *et al.*, 1988). However, the lack of survival of inoculant fungi at some sites may partly explain why inoculation with effective ectomycorrhizal isolates has not always increased the growth of trees in the field (Castellano & Trappe, 1991).

CONCLUSIONS

Inoculation with *Pisolithus* generally improved the early growth (less than 2 years) of eucalypts planted in marginal sites in the Philippines. Generally, the Australian isolate stimulated greater wood volume production in Pangasinan and in Surigao than the Philippine isolate and it persisted in the field under a range of environmental conditions. In Bukidnon, however, the two isolates of *Pisolithus* were equally effective in promoting wood volume of *E. urophylla* trees. Future work is required to test a wide range of ectomycorrhizal fungi, to monitor responses of eucalypts to inoculation over a longer period and to develop markers for easier monitoring of persistence of introduced fungi in the field. Although, the appearance of fruit bodies implies persistence, it does not always predict that mycelia from which they emanate had colonized the roots of nearby hosts.

ACKNOWLEDGMENTS

This work was funded by the Australian Centre for Inter-

national Agriculture Research (ACIAR Project 9044) and an Australian Agency for International Development (AusAID) postgraduate scholarship to the senior author. The senior author would like to thank AusAid for her attendance at this workshop. The authors thank the cooperators: Bukidnon Forest Industries, Paper Industries Corporation of the Philippines and the Department of Environment and Natural Resources Region 1, Dr. Brett Thomson and colleagues at BIOTECH for their assistance during the nursery and field phases of the project.

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