NODULATION OF THE LEGUME *PTEROCARPUS INDICUS* BY DIVERSE STRAINS OF RHIZOBIA

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LOK, E. H., O'HARA, G. & DELL, B. 2006. Nodulation of the legume *Pterocarpus indicus* by diverse strains of rhizobia. *Pterocarpus indicus* (Leguminosae: Papilionoideae) is a tropical legume with potential for commercial forest plantation. A glasshouse experiment was undertaken to investigate the effects of inoculation of 18 strains of diverse rhizobia comprising species from four genera on *P. indicus* seedlings. *Pterocarpus indicus* was effectively nodulated by *Bradyrhizobium elkani* WSM 2096. These seedlings had a significantly greater plant yield compared with seedlings with other strains and the controls. Strain *Bradyrhizobium* sp. WSM 3712, originally isolated from P. *indicus* from Malaysia, formed many partially effective nodules. However, three other slow-growing strains, *B. liaoningense* WSM 2098, *Bradyrhizobium* sp. TAL 643 and *Bradyrhizobium* sp. TAL 651, ineffectively nodulated *P. indicus*. The moderately fast-growing strain, *Mesorhizobium ciceri* WSM 2100, and four fast-growing strains, *Sinorhizobium meliloti* WSM 2114, *Rhizobium hainanense* WSM 2106, *Rhizobium gallicum* R 602 and *Rhizobium tropici* WSM 2110, also formed ineffective nodules on *P. indicus*. *Pterocarpus indicus* appears to be a diverse and promiscuous host for nodulation but with a narrow range for effective N_2-fixing symbiosis. This information is useful for future inoculation programmes for *P. indicus* in containerized nurseries.

Keywords: Inoculation, effective nodules, diverse, promiscuous host, narrow range

LOK, E. H., O'HARA, G. & DELL, B. 2006. Pembintilan kekacang *Pterocarpus indicus* oleh pelbagai jenis rizobium. *Pterocarpus indicus* (Leguminosae: Papilionoideae) ialah kekacang tropika yang mempunyai potensi dalam ladang hutan komersial. Satu uji kaji rumah kaca dijalankan untuk menyelidiki kesan inokulasi 18 jenis rizobium daripada empat genera terhadap anak benih *P. indicus*. *Pterocarpus indicus* menunjukkan pembintilan lengkap apabila diinokulasi dengan *Bradyrhizobium elkani* WSM 2096. Anak benih yang diinokulasi dengan WSM 2096 menunjukkan kadar pertumbuhan yang lebih cepat berbanding anak benih yang diinokulasi dengan jenis rizobium lain dan kawalan. Jenis rizobium *Bradyrhizobium* sp. WSM 3712 yang asalnya diasingkan daripada *P. indicus* dari Malaysia menunjukkan pembintilan separa efektif. Bagaimanapun, tiga jenis rizobium lain yang mempunyai kadar pertumbuhan lambat, *B. liaoningense* WSM 2098, *Bradyrhizobium* sp. TAL 643 dan *Bradyrhizobium* sp. TAL 651 tidak menghasilkan pembintilan efektif pada *P. indicus*. Jenis rizobium yang kadar pertumbuhannya sederhana iaitu *Mesorhizobium ciceri* WSM 2100 dan empat jenis rizobium yang kadar pertumbuhannya cepat iaitu *Sinorhizobium melilti* WSM 2114, *Rhizobium hainanense* WSM 2106, *Rhizobium gallicum* R 602 dan *Rhizobium tropici* WSM 2110 juga tidak menghasilkan pembintilan efektif pada *P. indicus*. Secara amnya, *P. indicus* boleh menjadi perumah kepada pelbagai jenis rizobium tetapi peranannya agak terhad bagi ikatan nitrogen yang berkesan. Maklumat ini dapat digunakan dalam program inokulasi *P. indicus* di peringkat tapak semaian pada masa hadapan.

Introduction

Pterocarpus indicus (Leguminosae: Papilionoideae) is one of the commercial tree legume species that dominate South-East Asia and some Pacific regions (Soerianegara & Lemmens 1993). This species has many common names, such as angsana or sena in Malaysia and Singapore, sonokembang in Indonesia and narra in the Philippines (Corner 1988). *Pterocarpus indicus* grows on a variety of soil types from fertile agricultural soil to rocky soil, along inundated river banks, swamps and lagoons (Allen & Allen 1981, Corner 1988). It has the status of national tree in the Philippines and has been identified by the Forest Research Institute Malaysia (FRIM) as one of the potential 'millennium tree' species for forest plantation establishment in Peninsular Malaysia because of its fast growth and other desirable characteristics (Appanah & Wienland 1993, Lok 1996). However, growth data are restricted to early reports from reforestation projects in the

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Philippines (Sardina 1951, Assidao & Cerna 1960) and individual amenity trees in Singapore (Wong 1982). *Pterocarpus indicus* is now mostly grown as an ornamental tree and is relatively rare in forests in South-East Asia due to extensive selective logging (Soerianegara & Lemmens 1993). The timber of *P. indicus* is classified as light hardwood and is used for light to heavy construction, joists, beams and interior finishes. The wood, which is commonly traded as rosewood, has beautiful distinct growth rings and is ranked among the finest for furniture making, high grade cabinet work, carvings, decorative flooring and musical instruments (Appanah & Weinland 1993, Soerianegara & Lemmens 1993).

Legumes, especially tropical woody species, are important for maintaining ecosystem fertility and are used in soil stabilization and revegetation programmes (Langkamp *et al.* 1979, Dreyfus & Dommergues 1981). *Pterocarpus* species are reported to nodulate with *Rhizobium* and *Bradyrhizobium* in the field but little is known about the specificity, symbiotic relationships and capacity to fix nitrogen in *P. indicus* (Allen & Allen 1981, Sprent 2001). Inoculation with N_2-fixing bacteria is an advantage where soil populations of compatible microsymbionts are low or absent such as may occur for the rehabilitation of disturbed lands (De Faria *et al.* 1987, Ndiaye & Ganry 1997) and introduction of industrial plantations (Berger 1993, Hogberg & Alexander 1995).

Inoculation of tree legumes with effective strains of rhizobia may be necessary in commercial forest nurseries to increase N_2-fixation capability following out-planting to the field (Chee *et al.* 1989, Turk & Keyser 1992, Cheng *et al.* 2002, Perez-Fernandez & Lamont 2003). Since N deficiency is a major fertility constraint in agriculture and forestry in the tropics, there is an urgent need to exploit N_2-fixing tree legumes for sustained productivity. The aim of this study therefore was to investigate the ability of Malaysian *P. indicus* to form nodules with a diverse range of rhizobia and to identify effective strains with potential for application as inocula in the nursery or field.

MATERIALS AND METHODS

Experimental design

A complete randomized block design was used with 18 strains of rhizobia (Table 1) and two control treatments (C1—uninoculated without added inorganic N and C2—uninoculated plus inorganic N). Four replicates were established per treatment. Each replicate was a single pot (15 cm diameter × 17.5 cm depth) containing three *P. indicus* seedlings. The trial was carried out between September and December 2003 in an evaporative-cooled glasshouse at Murdoch University, Western Australia. The mean minimum and maximum temperatures (± SE) were 18 ± 2 °C and 30 ± 3 °C respectively.

Plant and soil material

Seeds were collected in February 2002 from mature *P. indicus* growing at the Forest Research Institute Malaysia (FRIM), Kepong and stored at room temperature. Before sowing, the seeds were surface sterilized in 70% (v/v) ethanol for 60 s, followed by 45 s in freshly prepared 4% (w/v) sodium hypochlorite, followed by six rinses in sterile distilled water. Seeds were sown in plastic trays (29 × 35 cm) containing yellow sand sterilized by autoclaving for 20 min at 121 °C. After one week, seedlings with open cotyledons and 5 cm radicles were transplanted to pots. The pots had been previously soaked overnight in 4% (w/v) sodium hypochlorite, rinsed with sterile deionised water, lined with sterile paper towels and filled with 3 kg N-free yellow sand at pH 6.0.

The yellow sand was obtained from virgin woodlands near Perth, Western Australia where it forms ridges parallel to the coast known as the Karrakatta series (Bettenay *et al.* 1960). The sand is associated with the Tamala Limestone and represents the decalcified remnants of a Pleistocene lime sand dune. The sand has a pH (H_2O) range from 5.3 to 6.0 (McArthur 1991) and has been used in various studies on plant nutrition and mycorrhizal responses (Bougher *et al.* 1990, Brundrett *et al.* 1996).

To remove traces of inorganic nitrogen, the sand filled pots were flushed twice with 2 litres of boiling water and allowed to drain to field capacity. The prepared pots were then covered with sterile cling plastic sheeting until ready for use. After transplanting and inoculation, all pots were given a pulse of 20 ml aqueous solution containing KNO_3 (5 g l^{-1}) and the soil surface was covered with hypochlorite-rinsed alkathene beads to reduce evaporation and to prevent contamination. Pots were watered with deionised water as required, and weekly with sterile complete nutrient solution (minus N) (Howieson *et al.* 1995). The nutrient solution contained (g l^{-1}): 0.31 $MgSO_4.7H_2O$, 0.21 KH_2PO_4, 0.44 K_2SO_4, 0.06 FeEDTA, 0.05 CaSO4 and trace elements (mg l^{-1}) 0.116 H_3BO_4, 0.0045 $Na_2MoO_4.2H_2O$, 0.134 $ZnSO_4.7H_2O$, 0.01 $MnSO_4.H_2O$, 0.03 $CoSO_4.H_2O$ and 0.03 $CuSO_4.5H_2O$. The nitrogen-fed control was supplied weekly with 5 ml of KNO_3 (5 g l^{-1}). Water and nutrients were supplied through a sterile white PVC tube inserted in the centre of each pot and covered with a plastic cap.

Rhizobia strains

The strains of rhizobia used are listed in Table 1. Strain WSM 3712 was isolated from a nodule of *P. indicus* collected in 2002 from a potted seedling growing in the FRIM's nursery and was stored over silica gel. The nodule was left to soak in distilled water for four hours, surfaced sterilized by immersion in 70% (v/v) ethanol for 45 s, and 4% (w/v) sodium hypochlorite ($NaHClO_4$) for 60 s then followed by six rinses of sterile distilled water (modified from Vincent 1970). The nodule was crushed in a drop of sterile water and the contents transferred by a sterilized wire loop onto yeast extract mannitol agar (YMA) (Howieson *et al.* 1988). The plates were incubated at 28 °C and observed daily for 14 days for colonies just appeared after 7 to 10 days' incubation.

Sixteen strains of WSM and TAL, obtained from the Centre for Rhizobium Studies (CRS) at Murdoch University, Australia were recovered from vacuum-dried ampoules using the CRS method (Howieson *et al.* 1988). The strain R602 was obtained from the International Rhizobium Laboratory (INRA) in France.

Inoculation

Rhizobia strains were grown at 28 °C on YMA plates to produce sufficient culture for inoculation. Fast-growing strains of *Rhizobium* and *Sinorhizobium* were incubated for 3–4 days, the medium-growing strains of *Mesorhizobium* for 4–5 days and the slow-growing strains of *Bradyrhizobium* for 7–10 days. These colonies were then washed from the Petri plates using sterile sucrose solution 1% (w/v) and a suspension of 10 ml was used to inoculate each pot. Seedlings were inoculated two days after transplanting using a sterile syringe to distribute the suspension evenly onto the soil surface around each seedling.

Harvest

Plants were harvested after three months when

Table 1 Strains of rhizobia used in the study

Strain No.	Species	Host plant	Source
TAL 643	*Bradyrhizobium* sp.	*Canavalia gladiata*	RRIM
TAL 648	*Bradyrhizobium* sp.	*Psophocarpus tetragonolobus*	RRIM
TAL 651	*Bradyrhizobium* sp.	*Calopogonium mucunoides*	RRIM
TAL 656	*Bradyrhizobium* sp.	*Pachyrhizus erosus*	RRIM
R 602	*Rhizobium gallicum*	*Phaseolus vulgaris*	INRA
WSM 2096	*Bradyrhizobium elkanii*	*Glycine max*	CRS
WSM 2097	*Bradyrhizobium japonicum*	*Glycine max*	CRS
WSM 2098	*Bradyrhizobium liaoningense*	*Glycine max*	CRS
WSM 2100	*Mesorhizobium ciceri*	*Cicer arietinum*	CRS
WSM 2105	*Rhizobium galegae*	*Galega* sp.	CRS
WSM 2106	*Rhizobium hainanense*	*Desmodium* sp.	CRS
WSM 2108	*Rhizobium leguminosarum*	*Pisum sativum*	CRS
WSM 2110	*Rhizobium tropici*	*Phaeseolus vulgaris*	CRS
WSM 2114	*Sinorhizobium meliloti*	*Medicago sativa*	CRS
WSM 2115	*Sinorhizobium saheli*	*Acacia senegal*	CRS
WSM 2116	*Sinorhizobium terangae*	*Acacia senegal*	CRS
WSM 2117	*Mesorhizobium loti*	*Lotus edulis*	CRS
WSM 3712	*Bradyrhizobium* sp.	*Pterocarpus indicus*	FRIM

CRS = Centre for Rhizobium Studies, INRA = International Rhizobium Laboratory (France), FRIM = Forest Research Institute Malaysia, RRIM = Rubber Research Institute of Malaysia there were visible differences in plant growth. Roots were extracted from the sand with running water and nodulation was assessed based on the number of nodules, size, colour and position on the roots (Howieson *et al.* 1995). The shoots were separated and oven dried at 70 °C to constant weight (48 hours). Nodules used for microscopic studies were fixed overnight at 4 °C in gluteraldhyde (3% v/v) and in phosphate (0.025 M) buffer before being dehydrated and embedded in Spurr's resin (Spurr 1969). Observations on nodules were made using stereo and compound microscopes.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using the program SPSS version 11.5. Data transformation was carried out where necessary before Duncan's multiple range test was used to identify differences between means at $p \leq 0.05$.

RESULTS

Nodulation in *Pterocarpus indicus*

The uninoculated controls remained nodule free indicating that soil sterilization was successful and that nodulation did not occur without inoculation. Nodules were present on roots of *P. indicus* inoculated with strains from all four genera, *Bradyrhizobium, Mesorhizobium, Sinorhizobium* and *Rhizobium* after three months. Ten strains (TAL 643, TAL 651, R 602, WSM 2096, WSM 2098, WSM 2100, WSM 2106, WSM 2110, WSM 2114 and WSM 3712) formed nodules (Table 2), whereas eight strains (TAL 648, TAL 656, WSM 2097, WSM 2105, WSM 2108, WSM 2115, WSM 2116 and WSM 2117) did not nodulate. *Pterocarpus indicus* inoculated with four slow-growing *Bradyrhizobium* strains (WSM 3712, TAL 643, WSM 2096 and TAL 651) formed greater mean number of nodules per seedling whereas plants inoculated with the medium-growing (*Mesorhizobium*) and fast-growing (*Rhizobium* and *Sinorhizobium*) strains formed low numbers of nodules (Table 2). Strain WSM 3712 was identified as a *Bradyrhizobium* based on its growth rate on YMA.

Nodule location

Plants inoculated with *Bradyrhizobium* sp. (WSM 3712), *B. elkanii* (WSM 2096) and *Bradyrhizobium* sp. (TAL 643) had nodules on both the tap and main lateral roots (Table 2, Figure 1a). The nodules formed on plants inoculated with other strains were confined to the collar region of the tap root. The larger nodules formed by WSM 3712 and WSM 2096 were pink internally (Table 2), whereas all nodules of the other strains were white. *Pterocarpus indicus* nodules were mainly globose, smooth (Figure 1a), internally aeschynomenoid according to Sprent (2001), with determinate growth of oblate form and the central infected tissue having few or no uninfected cells (astragaloid in the terminology of Corby 1971) (Figure 1b).

Table 2 Mean nodule number, nodulated seedlings, nodule distribution, size and colour of nodules obtained in *Pterocarpus indicus* seedlings

Strain	Mean number of nodules per nodulated seedling (± SE)	Nodulated seedlings (%)	Nodule distribution (%)			Nodule size (%)			Nodule colour
			A	B	C	L	M	S	
WSM 3712	25.3 ± 6.3	83	93	7	-	-	18	82	pink
TAL 643	17.3 ± 5.0	83	57	28	15	-	19	81	white
WSM 2096	19.0 ± 9.0	42	38	46	16	16	42	42	pink
TAL 651	16	8	100	-	-	-	-	100	white
WSM 2106	3	8	100	-	-	-	100	-	white
WSM 2114	2	8	100	-	-	-	100	-	white
WSM 2100	1	8	100	-	-	-	-	100	white
R 602	1	8	100	-	-	-	-	100	white
WSM 2098	1.0 ± 0.0	8	100	-	-	-	-	100	white
WSM 2110	1	8	100	-	-	-	-	100	white

A = 0–3 cm from root collar, B = 3–5 cm from root collar, C = > 5 cm from root collar
L = large (> 5 mm), M = medium (3–5 mm), S = small (< 3 mm)

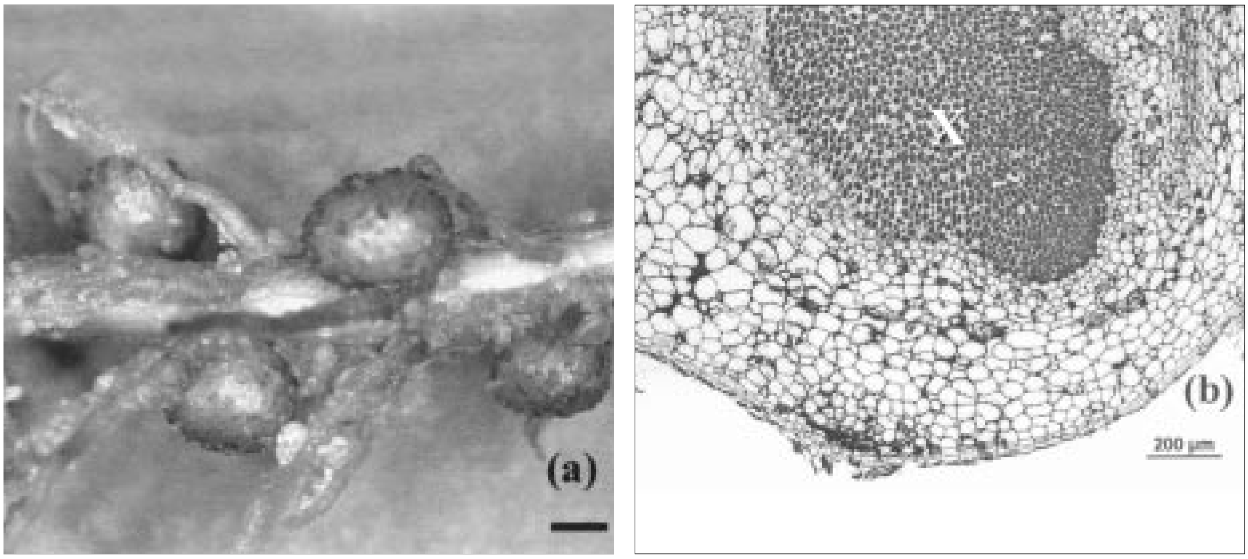

Figure 1 Morphology and anatomy of *Pterocarpus indicus* nodules formed with *Bradyrhizobium elkanii* (WSM 2096). (a) Typical morphology of aeschynomenoid nodules (Scale bar = 1 mm) (b) Transverse section of 3-month-old *P. indicus* nodule. The darker central stained region (X) shows infected tissues with determinate growth.

Dry matter production

Plants nodulated with *B. elkanii* (WSM 2096) or *Bradyrhizobium* sp. (WSM 3712) had dark green shoots compared with the uninoculated control plants without added inorganic N, but only WSM 2096 significantly increased shoot dry weight by the time of harvest (Figure 2). Plants inoculated with WSM 2096 and the uninoculated control supplied with inorganic N had similar shoot dry weights which were three times greater than the control without inorganic N (Figure 2).

DISCUSSION

Knowledge of rhizobia associated with *P. indicus* is limited. A significant finding from this study shows that *P. indicus* can be nodulated by diverse strains of rhizobia from four genera, i.e. *Bradyrhizobium, Rhizobium, Sinorhizobium* and *Mesorhizobium.* Nodulation of *P. indicus* by slow-growing *Bradyrhizobium* has been previously reported (Lim 1976, Allen & Allen 1981, Oyaizu *et al.* 1993) but this is the first report of nodulation of *P. indicus* by *Mesorhizobium, Rhizobium* and *Sinorhizobium.* In this study, *P. indicus* formed root nodules with seven different species of rhizobia. This study provides strong evidence to support the suggestion by Sprent and Parsons (2000) that

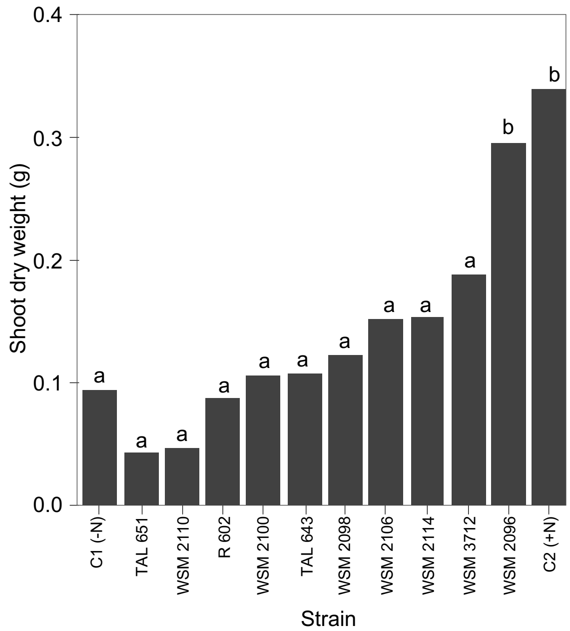

Figure 2 Mean shoot dry weight of *Pterocarpus indicus* seedlings. Bars with the same letter are not significantly different ($p \leq 0.05$).

nodulating species of *Pterocarpus*, such as *P. indicus*, are promiscuous tree legumes. Symbiotic promiscuity may be a benefit for legumes growing in natural ecosystems but could also present problems for inoculation with improved strains in the nursery or field.

Previous studies have reported mainly on the ability of *P. indicus* to nodulate with indigenous rhizobia in many soils in different countries but little has been reported concerning the effectiveness of these symbiotic relationships (De Faria *et al.* 1989, Sprent 1995, Moreira *et al.* 1998, Sprent & Parsons 2000). Only one of the 18 strains tested in this study was effective for N_2-fixation with *P. indicus*. *Bradyrhizobium elkanii* WSM 2096 formed pink nodules and significantly increased shoot dry weight. *Pterocarpus indicus* was previously reported by Allen and Allen (1981) to be effectively nodulated by strains of *Bradyrhizobium* that were also effective on *Vigna sinensis* (cowpea). By contrast in the present study the most effective strain was *B. elkanii* WSM 2096, a strain originally isolated from *Glycine max* (soybean).

In general, the morphological characteristics of nodules are closely related to the host tree species. For example, *Albizia paraserianthes* produces semi-globose nodules that are single or clustered while those in *Acacia auriculiformis* are globose to elongate and lobed. In this study, all nodules on *P. indicus* were found to be globose, single and of the aeschynomenoid type that are short-lived and similar to those reported earlier by Lim (1976), Allen & Allen (1981), Corby (1988) and Sprent (2001).

Further inoculation studies with a wide range of strains of rhizobia, including those that are isolated from taxonomically related species such as in *Dalbergia*, are required to find effective strains with potential for use as inocula. Whether woody legumes require inoculation in the nursery depends on the availability of compatible and effective rhizobia in the soils where they are to be out-planted. This should be investigated with any new tree species. The current study indicates that *P. indicus* is a promiscuous host with the ability to form symbioses with both slow and fast growing rhizobia. However the results also show that this tree legume may have a very narrow range for forming a highly effective N_2-fixing symbiosis with rhizobia.

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REFERENCES

Allen, O. N. & Allen, E. K. 1981. *The Leguminosae: a Source Book of Characteristics, Uses and Nodulation.* University of Wisconsin Press, Madison.

Appanah, S. & Weinland, G. W. 1993. *Planting Quality Timber Trees in Peninsular Malaysia: a Review.* Malayan Forest Record No. 38. Forest Research Institute Malaysia, Kepong.

Assidao, F. & Cerna, De La P. 1960. The growth of young narra trees in plantation Camp7 Minglanilla, Cebu. *Philippines Journal of Forestry* 16: 7–14.

Berger, J.I. 1993. Ecological restoration and non-indigeneous plant species: a review. *Restoration Ecology* 1: 74–82.

Bettenay, E., McArthur, W. M. & Hingston, F. J. 1960. *The Soil Associations of Part of the Swan Coastal Plain, Western Australia.* CSIRO, Perth.

Bougher, N. L., Grove, T. S. & Malajczuk, N. 1990. Growth and phosphorus acquisition of karri (*Eucalyptus diversicolor* F. Muell.) seedlings inoculated with ectomycorrhizal fungi in relation to phosphorus supply. *New Phytologist* 114: 77–85.

Brundrett, M., Dell, B., Malajczuk, N. & Gong, M. (Eds.). 1996. *Mycorrhizas for Plantation Forestry in Asia.* ACIAR Proceedings No. 62. ACIAR, Canberra.

Chee, C. W., Sundram, J., Date, R. A. & Roughley, R. J. 1989. Nodulation of *Leucaena leucocephala* in acid soils of Peninsular Malaysia. *Tropical Grasslands* 23: 171–178.

Cheng, Y., Watkin, E. L. J., O'Hara, G. W. & Howieson, J. G. 2002. *Medicago sativa* and *Medicago murex* differ in the nodulation response to soil acidity. *Plant and Soil* 238: 31–39.

Corby, H. D. L. 1971. The shape of leguminous nodules and the colour of leguminous roots. In Lie, T. A. & Mulder, E. G. (Eds.) Biological Nitrogen Fixation in Natural and Agricultural Habitats. *Plant and Soil* (special volume) 305.

Corby, H. D. L. 1988. Types of rhizobial nodules and their distribution among the leguminosae. *Kirkia* 13(1): 53–123.

Corner, E. J. H. 1988. *Wayside Trees of Malaysia.* Volume 1. Third edition. The Malayan Nature Society, Kuala Lumpur.

De Faria, S. M., De Lima, H. C., Franco, A. A., Mucci, E. S. F. & Sprent, J. I. 1987. Nodulation of legume trees from south east Brazil. *Plant and Soil* 99: 347–356.

De Faria, S. M., Lewis, G. P., Sprent, J. I. & Sutherland, J. M. 1989. Occurrence of nodulation in the Leguminosae. *New Phytologist* 111: 607–619.

Dreyfus, B. C. & Dommergues, Y. 1981. Nodulation of *Acacia* species by fast and slow-growing strains of *Rhizobium. Applied and Environmental Microbiology* 41: 97–99.

Hogberg, P. & Alexander, I. J. 1995. Roles of symbioses in African woodland and forest: evidence from ^{15}N abundance and foliar analysis. *Journal of Ecology* 83: 217–224.

Howieson, J. G., Ewing, M. A. & D'Antuono, M. F. 1988. Selection of acid tolerance in *Rhizobium meliloti. Plant and Soil* 105: 179–188.

Howieson, J. G., Loi, A. & Carr, S. J. 1995. *Biserrula pelecinus* L.— legume pasture species with potential for acid, duplex soils which is nodulated by unique root-nodule bacteria. *Australian Journal of Agriculture Research* 46: 997–1009.

Langkamp, P. J., Swinden, L. B. & Dalling, M. J. 1979. Nitrogen fixation (acetylene reduction) by *Acacia holocericea* on areas restored after mining at Groote Eylandt, Northern Territory. *Australian Journal of Botany* 27: 353–361.

Lok, E. H. 1996. The branching behaviour and silvicultural potential of *Pterocarpus indicus* using small cuttings. MSc thesis, Universiti Pertanian Malaysia, Serdang.

Lim, G. 1976. Rhizobium and nodulation of legumes in Singapore. Pp. 159–175 in Broughton, *et al.* (Eds.) *Proceedings of Symposium on Soil Microbiology and Plant Nutrition.* Kuala Lumpur.

McArthur, W. M. 1991. *Reference Soils of South-Western Australia.* Department of Agriculture, Perth.

Moreira, F. M. S., Haukka, K. & Young, J. P. W. 1998. Biodiversity of rhizobia isolated from a wide range of forest legumes in Brazil. *Molecular Ecology* 7: 889–895.

Ndiaye, M. & Ganry, F. 1997. Variation in the biological N_2 fixation by tree legumes in three ecological zones from the north to the south of Senegal. *Arid Soil Research* and *Rehabilitation* 11: 245–254.

Oyaizu, H., Matsumoto, S., Minamisawa, K. & Gamou, T. 1993. Distribution of rhizobia in leguminous plants surveyed by phylogenetic identification. *Journal of General Applied Microbiology* 39: 339–354.

Perez-Fernandez, M. A. & Lamont, B. B. 2003. Nodulation and performance of exotic and native legumes in Australian soils. *Australian Journal of Botany* 51: 543–553.

Sardina, A. C. 1951. The effect of length of narra cuttings on the percentage of survival. *Philippines Journal of Forestry* 14: 59–66.

Soerianegara, L. & Lemmens, R. H. M. J. (Eds.). 1993. *Plant Resources of South East-Asia No 5(1). Timber Trees: Major Commercial Timbers.* Pudoc Scientific Publishers, Wageningen.

Sprent, J. I. 2001. *Nodulation in Legumes.* Royal Botanic Gardens, Kew.

Sprent, J. I. 1995. Legume trees and shrubs in the tropics: N_2 fixation in perspective. *Soil Biology and Biochemistry* 27(4/5): 401–407.

Sprent, J. I. & Parsons, R. 2000. Nitrogen fixation in legume and non-legume trees. *Field Crops Research* 65: 183–196.

Spurr, A. R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* 26: 31.

Turk, D. & Keyser, H. H. 1992. Rhizobia that nodulate tree legumes: specificity of the host for nodulation and effectiveness. *Canada Journal of Microbiology* 38: 451–460.

Vincent, J. M. 1970. *A Manual for the Practical Study of Root-Nodule Bacteria.* International Biological Program Hand Book No. 15. Blackwell, Oxford.

Wong, T. M. 1982. Horticultural notes on the angsana (*Pterocarpus indicus* Willd.). *Gardens Bulletin of Singapore* 34: 189–202.