Nutrition and nitrogen-fixation in Malaysian *Pterocarpus indicus* Willd.

By

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DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Abstract

Pterocarpus indicus is a promising tropical woody legume for the establishment of forest plantations in Malaysia. Woody legumes that form symbiotic relationships with nitrogen-fixing bacteria also play an important role for forest restoration on degraded land. Although *P. indicus* has been widely planted as an amenity tree in SE Asia, its silvicultural requirements have not been determined. There are no recommendations for fertilizer or for inoculation with nitrogen-fixing bacteria. This thesis explores the phosphorus (P) and nitrogen (N) requirements of seedlings and identifies a range of nitrogen-fixing bacteria capable of forming root nodules under glasshouse conditions.

Four glasshouse experiments were undertaken on two soil types: Yalanbee sandy gravel (YB) and yellow sand (YS) to determine the P and N concentration ranges in the foliage of deficient and healthy plants and to define critical nutrient concentrations for the diagnosis of deficiency. There was a narrow range in rates of P fertilizer, supplied as aerophos, $Ca(H_2PO_4)_2.H_2O$, between deficiency and toxicity in both soil types. The relationship between yield and P concentration in the youngest fully expanded leaf (YFEL) enabled critical P concentrations for the diagnosis of deficiency (0.17%) and toxicity (0.41%) to be determined at 90% maximum yield from linear regressions fitted to the data. The foliar P concentration ranges for deficiency and toxicity were similar to other nitrogen-fixing trees. Only plants in YS responded to inorganic N fertilizer, and soil analysis suggested that seedlings may take up ammonium-N in preference to nitrate-N at luxury supply. A critical concentration for the

diagnosis of N deficiency was not able to be determined due to the lack of data points. Plants with adequate N fertilizer had YFEL N concentrations of 2-3.5% dry weight.

To determine whether *P. indicus* is more sensitive to luxury soil P supply than other fast-growing legume trees, the P response of *P. indicus* in YB was compared with three other woody legumes, *Pterocarpus macrocarpus*, *Acacia mangium* and *Sesbania formosa*. The sensitivity of *P. indicus* to high P was confirmed and the response shown to be similar to *P. macrocarpus*. Both species showed severe symptoms of P toxicity, namely leaf necrosis and stunted growth. In contrast, shoot and root yields of *A. mangium* and *S. formosa* were not reduced at luxury P supply and yield x fertilizer relationships were able to be fitted to the Mitscherlich model. Critical P concentrations for the diagnosis of P deficiency in *A. mangium* and *S. formosa*, derived using the Mitscherlich model, were estimated to be 0.2-0.3% dry weight for the YFELs.

Two glasshouse inoculation trials were carried out using diverse strains of root nodule bacteria in order to identify strains suitable for inoculation in the nursery. There were eight strains from *Bradyrhizobium*, five from *Rhizobium*, three from *Sinorhizobium* and two *Mesorhizobium* strains. *P. indicus* formed nodules with strains from *Bradyrhizobium*, *Rhizobium*, *Sinorhizobium* and *Mesorhizobium*, which suggests it is a promiscuous host. Nodules formed were globose, single and of aeschynomenoid type. In the first trial, *Bradyrhizobium* strain WSM 2096, promoted shoot growth while in the second trial, *Bradyrhizobium* strain WSM 3712 promoted shoot growth. Growth

stimulation was similar to the uninoculated control supplied with inorganic N as KNO_3 but was inferior to plants given $(NH_4)_2SO_4$.

The response of *P. indicus* to low soil P in inoculated and uninoculated plants was studied in a pot trial comprising two P treatments (nil, just adequate). Plants were grown for 3 and 6 weeks. At nil fertilizer P, uninoculated *P. indicus* seedlings had higher total root length and root dry weight than those with adequate P. Inoculation with WSM 3712 suppressed root growth relative to uninoculated plants.

Information gathered in this thesis has application for the production of planting stock in forest nurseries. Firstly, care needs to be taken to ensure that rates of P supplied as hard or liquid fertilizer are not in the range likely to cause toxicity. Secondly, if any starter inorganic N fertilizer is to be used then it should be supplied either as ammonium-N or as urea. Thirdly, more research is required in order to identify effective strains of rhizobia for widespread commercial application. Fourthly, the critical foliar P concentrations identified for the diagnosis of P deficiency and P toxicity can be used to help interpret foliar analysis of seedlings in the future.

Publications arising from this research

Lok E.H., O'Hara, G.W. and Dell, B. 2006. Nodulation of the legume *Pterocarpus indicus* Willd. by diverse strains of rhizobia. *Journal of Tropical Forest Science* 18(3): 188-194.

It is anticipated that further publications will arise from this work.

List of abbreviations

ANOVA	Analysis of variance
CRS	Centre for Rhizobium Studies at Murdoch University
DBH	Diameter at breast height (1.3 m)
DMRT	Duncan's New Multiple Range test
FRIM	Forest Research Institute Malaysia
INRA	Institute national de la Recherche Agronomique, France
N	Nitrogen
n	Number of samples
P	Phosphorus
PCR	Polymerase chain reaction
R	Rhizobium
R^2	Correlated regression coefficients
RCBD	Randomized complete block design
S.E.	Standard error
Sp.	Species (singular; spp., plural)
T	Temperature (^oC)
WA	Western Australia
WSM	Western Australia Soil Microbiology
YB	Yalanbee soil
YFEL	Youngest fully expanded leaf
YMA	Yeast mannitol agar medium
YS	Yellow sand

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