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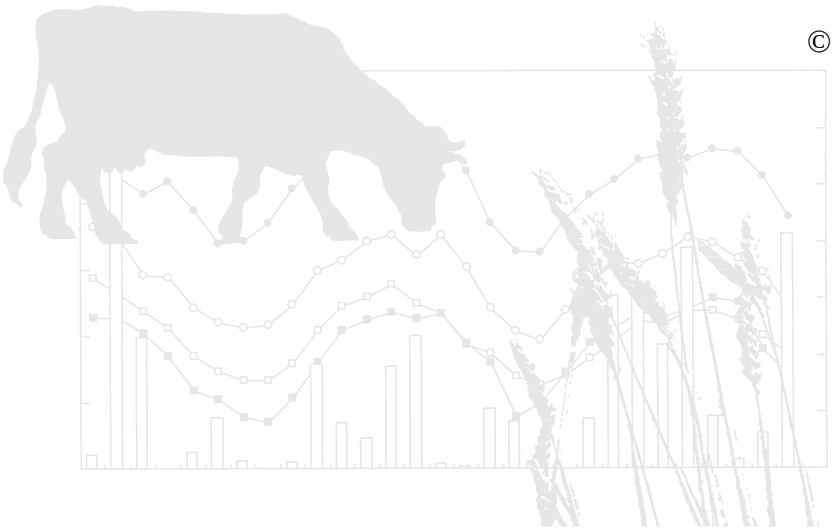

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Factors affecting the early growth of *Leucaena leucocephala*
2. Importance of arbuscular mycorrhizal fungi, grass competition and phosphorus application on yield and nodulation of leucaena in pots

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Summary. Slow seedling growth is a limitation to the more widespread adoption of the tree legume, leucaena (*Leucaena leucocephala*). Three glasshouse trials examined the role of arbuscular mycorrhiza and phosphorus (P) nutrition in determining early growth and nodulation of leucaena. Treatments included soil types, inoculation with arbuscular mycorrhizal (AM) fungi, P application, grass competition and fumigation with methyl bromide, an anti-fungal agent. Plant measurements included colonisation by AM fungi, nodule weight, tissue nitrogen (N) and P concentrations.

Slower early growth of leucaena in a soil from Mt Cotton than in soils from Gayndah or Theodore was due to slow colonisation of roots by AM fungi. Sequential harvests of plants revealed that rate of colonisation in the Mt Cotton soil was only half that in the Theodore soil prior to 28 days after planting resulting in subcritical P concentrations 21 days after sowing and an approximate halving of top dry weight 41 days after sowing. However, following increased infection, tissue P concentration and final plant growth 98 days after sowing were similar in both soils.

Early seedling growth in the Mt Cotton soil was increased by inoculating the soil with mulch containing AM fungi but not with soil collected from beneath established leucaena added at a lower rate. Phosphorus application significantly increased growth of leucaena seedlings, but only the highest rate of 1200 kg P/ha was able to prevent early P deficiency. Final growth was reduced by 50% in the presence of *Panicum maximum* as a result of increased competition for N and P and by 90% in fumigated soil as a result of P deficiency.

The results of these experiments confirm the important role of AM fungi on early seedling growth of leucaena. However, the potential to increase early growth using a soil or mulch inoculum containing AM fungi or P fertiliser may be limited by the high rates of application needed. More work is needed to determine whether slow rate of infection is a significant limitation in soils other than the Mt Cotton soil in the field.

Introduction

Poor establishment has been identified as one of the major limitations to the more widespread adoption of leucaena (Wildin 1982). In previous field trials (Brandon and Shelton 1997*a*), first year yield was found to be strongly influenced by phosphorus (P) nutrition. Leucaena grown at Mt Cotton (5 mg bicarbonate-extractable P/kg) responded to large applications of P fertiliser, while seedlings at Theodore and Gayndah (10 and 35 mg P/kg respectively) did not.

Ruaysoongnern (1990) found that hyphae of naturally occurring arbuscular mycorrhizal (AM) fungi were important in determining the efficiency with which leucaena could extract P from the soil. Where infection by this fungi was low or absent due to fumigation of the soils with methyl bromide, plants responded to higher than normal application of P. Leucaena may be particularly dependent on the fungal symbiosis due to its coarse root system and few root hairs (Yost 1981); structures normally associated with efficient P uptake.

We aimed to examine the role of colonisation by AM fungi and P nutrition on early growth and nodulation of leucaena in soils used previously in field trials. Effects of grass competition, application of P fertiliser, inoculation with AM fungi and fumigation of the soil with methyl bromide were also examined.

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Materials and methods

Experiment 1. Importance of AM fungi on growth of leucaena in three soils

Soils. The Mt Cotton soil came from a recently cleared area of the University of Queensland farm (27°37'S) at Mt Cotton. It was a gleyed podzolic with 5 mg P/kg bicarbonate-extractable P and pH 5.9 (1 : 5 H_2O) in the top 15 cm. The Theodore soil came from an area of improved pasture from the Brigalow Research Station near Theodore (24°42'S) and was a solodic soil with 10 mg P/kg bicarbonate-extractable P and pH 7.3 (1 : 5 H_2O) in the top 15 cm.

The Gayndah soil came from a previously cropped area of the Brian Pastures Research Station near Gayndah (25°39'S) and was a black earth with 35 mg P/kg bicarbonate-extractable P and pH 6.9 (1:5 H_2O) in the top 15 cm.

Treatments. Treatments applied to each of the 3 soils included 2 rates of P and 2 mycorrhizal treatments applied in factorial combination. Rates of P were equivalent to 100 (P100) and 1200 kg P/ha (P1200). The mycorrhizal treatments were: (i) M_1, natural soil to which a sterilised mycorrhizal inoculum was added; and (ii) M_2, natural soil to which a non-sterilised mycorrhizal inoculum was added. The mycorrhizal inoculum consisted of a mixture of mulch collected from the 0–5 cm layer of the soil from beneath established stands of leucaena at Mt Cotton, Theodore and Gayndah. There were 3 replications arranged in a block design.

Method. Natural topsoil (0–15 cm) from each of the sites was passed through a 1 cm sieve and 3.5 kg air-dried soil was weighed into 20-cm-diameter pots lined with polyethylene bags. The AM fungal inoculum was added at 180 g/pot and mixed through the soil during potting. An equivalent amount of sterilised inoculum (steamed for 1 h) was added to pots containing steam sterilised or non-inoculated natural soil.

Lime was incorporated into soil collected from the Mt Cotton and Brigalow sites during potting at a rate equivalent to 2.4 and 1.7 t/ha respectively. Appropriate amounts of P as double superphosphate were also incorporated into all soils before sowing. The basal nutrients, sulfur (60 kg S/ha), potassium (60 kg K/ha), magnesium (25 kg Mg/ha), zinc (2.5 kg Zn/ha), copper (2 kg Cu/ha), boron (0.6 kg B/ha) and molybdenum (0.2 kg Mo/ha), were applied in solution to all pots before sowing.

Seed of leucaena (*Leucaena leucocephala*) cv. Cunningham was scarified in boiling water for 3–4 s and inoculated with *Rhizobium* strain CB81. Twelve seeds/pot were sown at 1–1.5 cm depth. Pots were watered to field capacity as estimated by weight of soil following 24 h free drainage. Plants were thinned to 4/pot by day 20. Pots were randomised within blocks weekly.

Measurements. Youngest fully expanded leaves were sampled 10 weeks after sowing and dried at 60°C, ground, digested using Kjeldahl digestion and analysed for P. Remaining shoot material was harvested by cutting plants at ground level, dried at 60°C for 48 h and weighed.

Soil was removed from the roots of plants in the M_1 and M_2 treatments by gentle washing above a 0.5 cm sieve. A root sample was assessed for colonisation by AM fungi using the method of Phillips and Hayman (1970). Root colonisation was determined using a grid-line intersection method (Giovannetti and Mosse 1980) and results expressed as a percentage of total root length infected by AM fungi.

Experiment 2. Effects of soil type, fumigation, inoculation with AM fungi and grass competition

Treatments. Leucaena was grown in: (i) natural soil from Mt Cotton; (ii) natural soil from Theodore; (iii) natural soil from Mt Cotton to which an AM fungal inoculum had been added; (iv) soil from Mt Cotton fumigated with methyl bromide to kill AM fungi; and (v) natural soil from Mt Cotton in the presence of the tropical grass *Panicum maximum*. Treatments were repeated 3 times and arranged within blocks. There were 36 pots of each treatment to allow for 12 destructive sequential harvests.

Method. Natural topsoil (0–15 cm) from each of the sites was passed through a 1 cm sieve and 8 kg air-dried soil was weighed into 30-cm-diameter pots lined with plastic. Treatments of soil inoculation with AM fungi and soil fumigation with methyl bromide were applied before sowing.

The AM fungal inoculum consisted of equal quantities of soil collected from beneath established stands of leucaena at 3 sites in south-east Queensland (Mt Cotton, Theodore, Gayndah). Inoculum was added at 100 g/pot and mixed through the soil. An equivalent amount of sterilised inoculum (autoclaved for 1 h) was added to pots containing non-inoculated natural soil. Pots to be fumigated were placed beneath a double layer of plastic sheeting and treated with methyl bromide at 680 g/m^2.

Basal nutrients of 25 kg N/ha (on a surface area basis), 70 kg K/ha, 5 kg Zn/ha, 3 kg Cu/ha, 0.25 kg cobalt/ha, 100 kg Mg/ha, 0.5 kg B/ha and 0.5 kg Mo/ha were applied in solution to all pots. Phosphorus was also applied to all treatments at 100 kg/ha in solution to mask differences in initial bicarbonate-extractable P levels between the soils. Lime was applied to the Mt Cotton soil at 3.6 t/ha and mixed through the soil before addition of nutrients in solution.

Seed of leucaena (*Leucaena leucocephala*) cv. Cunningham was scarified in hot water at 80°C for 3 min and inoculated with *Rhizobium* strain CB3060. Twenty seeds/pot were sown on 10 January 1989 in the inoculated and uninoculated pots. Sowing of fumigated

soil was delayed when the continued emergence of some weed seedlings in this treatment indicated that the fumigation may not have been totally effective. Pots were refumigated at the same rate and allowed to air for 1 week before being planted on 17 January. Harvests in this treatment were all delayed 1 week so that plants were an equivalent age to those in the non-fumigated treatments.

Three *Panicum maximum* seedlings were planted around the perimeter of the pot at the time of sowing leucaena in the grass competition treatment. After emergence, the leucaena plants were progressively thinned to 3/pot by 8 weeks after sowing. Progressive thinning of plants enabled some extra comparisons of tissue nutrient concentrations on days 7, 12, 16 and 23 after sowing. Pots were maintained at field capacity (determined by the pressure plate at 10 kPa) by watering to weight daily. *Panicum maximum* seedlings were regularly trimmed to 15 cm (about once every 3 weeks) to prevent shading of leucaena.

Measurements. Plant height was determined each week by measuring the distance from the soil level to the growing apex of each plant in the pot. This was done for 10 randomly selected plants in each treatment and replication until the last and second-last harvest when all remaining plants in each treatment were measured (3 and 6 plants/replication respectively). Destructive harvests for plant dry weight, tissue concentrations of P and N, and root colonisation by AM fungi were made 10, 14, 21, 28, 34, 41, 49, 55, 63, 69, 90 and 98 days after sowing. Leucaena shoots from all treatments were dried and weighed, and P and N concentrations were determined in an autoanalyser after Kjeldahl digestion. Additional shoots removed in the thinning process were analysed for N and P in the same way.

Tissue staining procedure and method of assessing colonisation by AM fungi were the same as for experiment 1. In the leucaena–*Panicum maximum* treatment, the combined root system was stained for AM fungal colonisation. Grass roots were generally damaged by the prestaining method used for leucaena roots, and colonisation was determined in leucaena roots only, which were easily distinguished under the microscope by their greater coarseness. Nodules in a subsample of roots were counted, removed, dried and weighed, and nodule weight for the entire root system was then calculated.

Experiment 3. Effect of phosphorus application

Treatments in this experiment consisted of 4 P levels applied to pots of the Mt Cotton soil at (kg/ha): 0, 20, 100 and 1200. Pots were arranged in 3 blocks and 3 pots/treatment harvested on days 14, 24, 34, 46, 56, 66, 85 and 98 after sowing. The 100 kg P/ha treatment was common to experiment 1 and was sown at the same time in the same glasshouse using the same blocking arrangement. However, because harvest intervals differed between the 2 experiments (12 harvests in experiment 2 and 8 in experiment 3), statistical comparisons could only be made for harvests on days 14, 34, 56 and 98 when the harvest dates coincided. All other procedures including soil collection, basal nutrient application, soil preparation and fumigation methods were the same as those for experiment 2.

Results

Experiment 1. Importance of AM fungi on growth of leucaena in three soils

Application of the AM inoculum increased growth of plants in the Mt Cotton soil fertilised at 100 kg P/ha but not plants grown at 1200 kg P/ha (Table 1). Colonisation levels 10 weeks after sowing were lower in the uninoculated plants at Mt Cotton (40%) than inoculated plants (66%). Levels of colonisation in the Theodore and Gayndah soils were similar in both inoculated and uninoculated treatments (63–70%) and there were no growth responses to application of the mycorrhizal inoculum or to P fertiliser at the higher rate. Tissue P concentrations in youngest fully expanded leaves were not significantly different between soils or treatments at final harvest and ranged from 0.36 to 0.40%.

Experiment 2. Effects of soil type, fumigation, inoculation with AM fungi and grass competition

Colonisation by AM fungi. Early rate of colonisation was almost twice as rapid in the Theodore soil as the Mt Cotton soil. In contrast to the results of the first experiment, inoculation with AM fungi did not increase rate of colonisation in the Mt Cotton soil and results of inoculated and uninoculated treatments were, therefore, averaged and presented together. Colonisation levels measured in leucaena growing with *Panicum maximum* were generally similar to those of plants grown alone.

Table 1. Dry matter yield of leucaena shoots (g/pot) grown in three soils with two phosphorus rates in the presence (M_2) and absence (M_1) of an AM inoculum

Mycorrhizal treatment	Phosphorus applied (kg P/ha) 100	1200	Mean
	Mt Cotton soil		
M_1	8.4	13.7	11.1
M_2	11.9	12.6	12.3
Mean	10.2	13.2	
	Theodore soil		
M_1	10.9	11.2	11.0
M_2	12.5	11.5	12.0
Mean	11.7	11.4	
	Gayndah soil		
M_1	13.0	11.0	12.0
M_2	11.2	10.9	11.0
Mean	12.1	11.0	

l.s.d. ($P = 0.05$)
Soil x phosphorus = 1.5; soil x mycorrhiza = 1.8

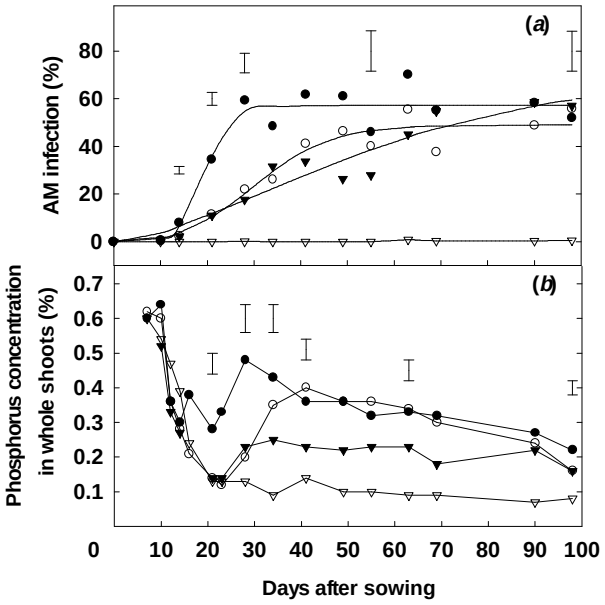

Figure 1. (*a*) Colonisation of leucaena roots by arbuscular mycorrhizal (AM) fungi and (*b*) tissue phosphorus concentrations in plant shoots in soil from: Mt Cotton (○); Theodore (●); Mt Cotton following fumigation with methyl bromide (∇); and Mt Cotton in the presence of *Panicum maximum* (▼). Logistical curves were fitted to AM fungi colonisation data ($r^2 > 0.6$). Vertical bars represent l.s.d. values at $P = 0.05$.

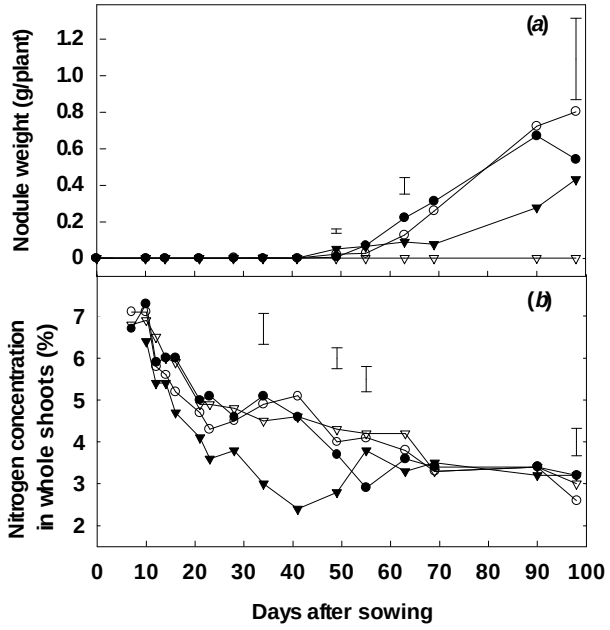

Figure 2. (*a*) Nodule weight and (*b*) tissue nitrogen concentrations in plant shoots in soil from: Mt Cotton (○); Theodore (●); Mt Cotton following fumigation with methyl bromide (∇); and Mt Cotton in the presence of *Panicum maximum* (▼). Vertical bars represent l.s.d. values at $P = 0.05$.

No colonisation was observed in soil fumigated with methyl bromide (Fig. 1*a*).

Tissue phosphorus concentration. Slower infection in the Mt Cotton soil resulted in lower concentrations of P in plant shoots than in the Theodore soil. Phosphorus concentration in plant shoots in the Mt Cotton soil 21 days after sowing was only 0.1% compared with >0.2% in the Theodore soil. However, following day 21, concentration in the non-fumigated Mt Cotton soil increased and concentrations were similar in both soils 42 days after sowing. Concentrations in plants growing in the fumigated Mt Cotton soil, however, remained at 0.1%. Concentrations of P in leucaena competing with *Panicum maximum* in the Mt Cotton soil increased after day 21 but remained at levels about mid-way between those of plants in fumigated and non-fumigated soil (Fig. 1*b*).

Total nodule weight. Nodules were first observed in the 2 soils at about the same time (21–28 days after sowing). Nodule weight reflected early growth in the various treatments (Fig. 2*a*) and was not significantly different in the 2 soils after day 49. Although small nodules formed in *Rhizobium*-inoculated leucaena growing in the fumigated treatment 21 days after sowing, they did not increase in size or number (Fig. 2*a*). *Panicum maximum* reduced final nodule weight by 50% (Fig. 2*a*) but this was not significant due to high variability in nodule weights between replications.

Tissue nitrogen concentration. Nitrogen concentration in whole plant shoots tended to decrease with time as plants increased in size and became progressively more woody. Concentrations were similar in both soils throughout the trial (Fig. 2*b*) but were significantly lower during early growth in plants competing with *Panicum maximum*.

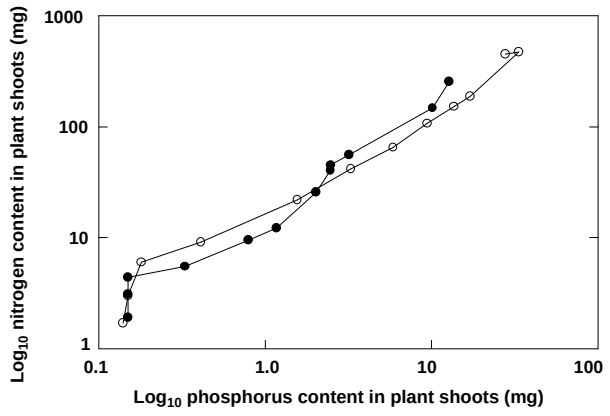

Figure 3. Nitrogen content of plant shoots plotted against phosphorus content of plant shoots of leucaena grown in the presence (●) and absence (○) of competition by *Panicum maximum*.

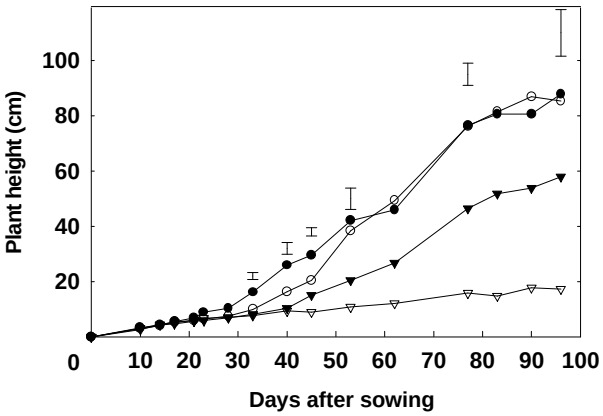

Figure 4. Plant height of leucaena growing in soil from: Mt Cotton (○); Theodore (●); Mt Cotton following fumigation with methyl bromide (∇); and Mt Cotton in the presence of *Panicum maximum* (▼). Vertical bars represent l.s.d. values at $P = 0.05$.

Nitrogen and phosphorus content. Nitrogen content was plotted against P content in plant shoots for plants growing in the presence and absence of *Panicum maximum* to give an indication of the relative rates of accumulation of N to P in plant shoots in these 2 treatments. Grass competition reduced concentration of N relative to P during early growth, but the reverse was true during later growth (Fig. 3).

Plant height

Plant height data is presented rather than shoot dry weight due to the greater precision in its measurement as a result of the larger number of plants measured for height than for dry weight. Plant height increased more slowly in the Mt Cotton soil than in the Theodore soil in the first 41 days after sowing (Fig. 4), but final plant height (and dry weight) were not significantly different between soils. Maximum difference in terms of height and plant dry weight occurred 41 days after sowing when height was 26 cm in the Theodore soil and 16 cm in the Mt Cotton soil, total plant dry weight was 2.6 g/plant in the Theodore soil and 1.3 g/plant in the Mt Cotton soil. Grass competition and soil fumigation greatly reduced plant height from day 41 (Fig. 4). Final shoot weight of leucaena was 20.2 g/plant in non-fumigated soil but was reduced by 50% by grass competition and 90% by soil fumigation.

Experiment 3. Effect of phosphorus application

Colonisation by AM fungi and tissue phosphorus concentration. Early colonisation of leucaena roots by AM fungi was significantly reduced by application of P at 1200 kg P/ha. Final colonisation levels, however, were similar at all P rates (Fig. 5*a*). Despite slower early colonisation, tissue P concentrations at 1200 kg P/ha were higher than at the lower 3 rates (Fig. 5*b*).

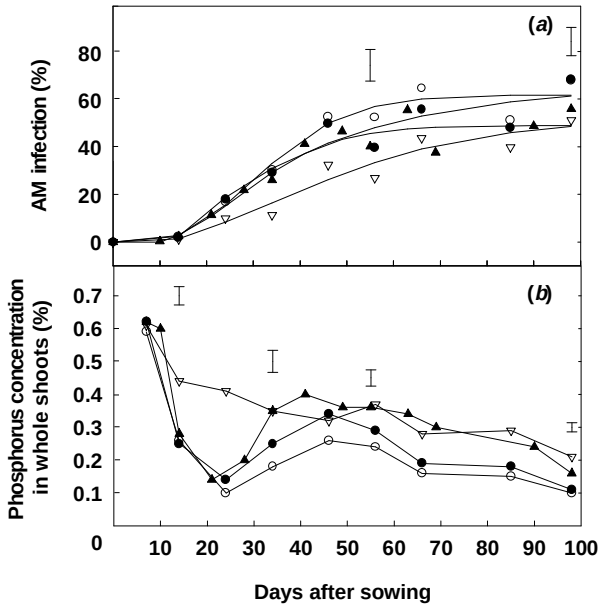

Figure 5. (*a*) Colonisation of leucaena roots by arbuscular mycorrhizal (AM) fungi and (*b*) tissue phosphorus concentrations in plant shoots in soil from: Mt Cotton following application of phosphorus at rates of 0 (○), 25 (●), 100 (▲), and 1200 kg/ha (∇). Logistical curves were fitted to AM colonisation data ($r^2 > 0.6$). Vertical bars represent l.s.d. values at $P = 0.05$.

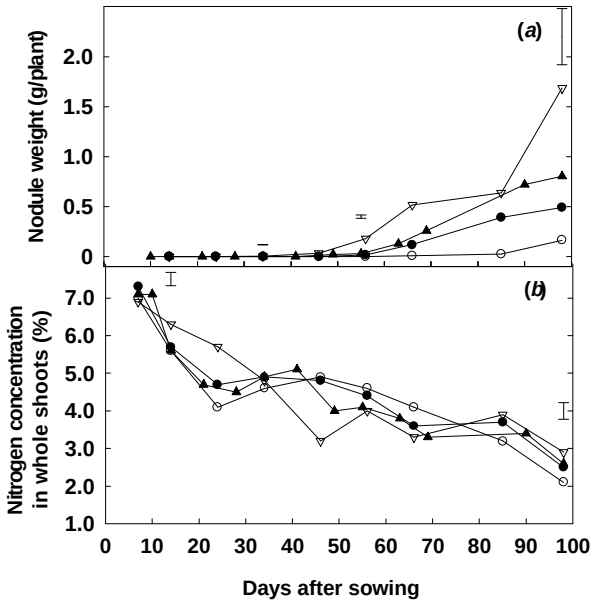

Figure 6. (*a*) Nodule weight and (*b*) tissue nitrogen concentrations in plant shoots in soil from Mt Cotton following application of phosphorus at rates of: 0 (○), 25 (●), 100 (▲), and 1200 kg/ha (∇). Vertical bars represent l.s.d. values at $P = 0.05$.

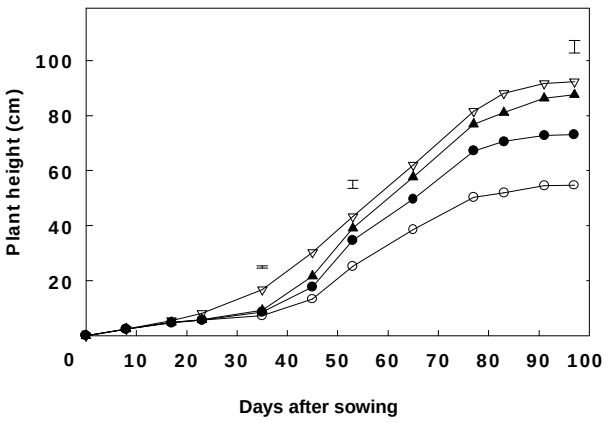

Figure 7. Plant height of leucaena growing in soil from Mt Cotton following application of phosphorus at rates of: 0 (○), 25 (●), 100 (▲), and 1200 kg/ha (∇). Vertical bars represent l.s.d. values at $P = 0.05$.

Nodule weight and nitrogen concentration. Phosphorus application increased nodule weight, and final nodule weight at 1200 kg P/ha was double that at the lower P rates (Fig. 6*a*). However, N concentration in plants fertilised at 1200 kg P/ha decreased more rapidly after 21 days of growth, reaching a minimum 46 days after sowing before increasing after day 46 along with a corresponding increase in nodule weight (Fig. 6*b*).

Plant height and dry weight. Application of 1200 kg P/ha was initially needed to maximise plant height (Fig. 7) and total plant dry weight at day 34 (1.20 g) was double that at the lower rates (0.35–0.57 g). Final dry weight of the 100 kg P/ha treatment following increased colonisation by AM fungi was about 90% that at the higher rate. Dry weight at the lower rates of 0 and 20 kg P/ha were about 50 and 70%, respectively.

Discussion

Phosphorus nutrition appeared to be the major factor influencing growth and nodulation of *Rhizobium*-inoculated leucaena under non-limiting water conditions. However, significant effects of colonisation on growth and nodulation of leucaena in non-fumigated soils only occurred during early seedling growth (<55 days) while effects of P application, grass competition and fumigation remained significant throughout the trial (>55 days).

Soil type

Differences in early rate of root colonisation by native populations of AM fungi in different soils were confirmed in this experiment. The development of subcritical P concentrations in leucaena growing in the natural Mt Cotton soil was similar to that described by Habte *et al.* (1987) for plants growing in fumigated soil and by Habte and Fox (1989) for leucaena growing in natural soils mixed with sterilised sand. The development of such low concentrations in non-sterilised soils have not been reported previously and confirm the major role of AM infection in determining early P nutrition of leucaena.

Although slow early colonisation and P deficiency reduced early seedling growth by 50%, there were no long-term effects on plant growth, and following continued colonisation, tissue P levels increased to non-limiting levels 41 days after sowing. This is consistent with the finding of Shepherd *et al.* (1996) that infection increased linearly 25–69 days after sowing in several soils and the observations by Manjunath and Habte (1988) that the uptake of P and other immobile nutrients closely paralleled that of colonisation 10–30 days after sowing.

Although differences caused by mycorrhizal infection in the current pot trial were generally confined to early seedling growth, we anticipated that larger effects would be observed in the field where in addition to P nutrition, seedlings must cope with other factors such as low moisture availability or weed competition. However, this was generally not found to be the case in subsequent field work, where colonisation by AM fungi was quickly able to meet plants' P requirements and environmental factors quickly became relatively more important in determining growth (Brandon and Shelton 1997*b*).

Reasons for the slower infection in the Mt Cotton soil compared with the Theodore soil include: (i) clearing of native vegetation and site disturbance; (ii) fallowing of the soil following time of clearing; and (iii) periodic waterlogging—all of which may reduce levels of AM fungi (Ilag *et al*. 1987; Thompson 1987; Evans and Miller 1990). Soil collected from this site at the time of this experiment may, therefore, have been abnormally low in AM fungal propagules. The rate of colonisation by indigenous AM fungi in a wider range of soils is examined in Brandon and Shelton (1997*b*).

Although there were significant differences in early nodule weights on plants growing in the Mt Cotton and Theodore soils, these appeared to be largely a reflection of differences in plant growth, and N concentration in the early part of growth generally remained above the critical level of 4.5% suggested by Ruaysoongnern *et al.* (1989). Similar P-mediated effects on nodulation caused by increased AM colonisation in leucaena have been reported by Manjunath *et al.* (1984).

Nodules were observed on plants grown in both the Mt Cotton and Theodore soils 21–28 days after sowing. This was much earlier than the 50 days observed by Bushby (1982). Despite formation of nodules early in the plant's growth, N deficiency occurred where grass competition reduced availability of soil N or where early growth of leucaena was extremely rapid as in the plants fertilised with 1200 kg P/ha. However, these effects were temporary and disappeared as nodule mass increased.

Soil fumigation

The almost complete dependence of leucaena on AM fungi for normal uptake of soil P was demonstrated by the 90% reduction in plant growth in the Mt Cotton soil following fumigation with methyl bromide. The unaided roots of leucaena were unable to extract sufficient P and the concentration in plant shoots decreased to 0.1% (Fig. 1*b*). This was well below the suggested critical concentration of 0.21% suggested by Ruaysoongnern *et al.* (1989) and similar to the 'starvation' level (0.08%) reported by Habte *et al.* (1987) for leucaena growing in fumigated soil. Unlike plants growing in non-fumigated soil, concentrations remained at this low level for the remainder of the trial.

The effect of soil fumigation did not appear to be due to effects on nodulation or nitrogen fixation. Nodules were first observed on *Rhizobium*-inoculated leucaena plants in the fumigated soil at about the same time as in non-fumigated soil. However, despite the formation of a small number of nodules, N applied at sowing (25 kg N/ha) probably supplied the plants with most of their N requirements and nodules did not increase in size or number.

Ruaysoongnern (1990) found that the negative effects of soil fumigation with methyl bromide on leucaena growth and nodulation could be reversed by reinoculation of the soil with AM fungi or by application of high rates of P. He concluded that the effects of fumigation were due to the prevention of colonisation by AM fungi rather than direct effects of methyl bromide on plant growth or nodulation.

Arbuscular mycorrhizal inoculation

Inoculation with AM fungi improved early seedling growth in Mt Cotton soil in experiment 1 but not in experiment 2. Inocula used differed, with mulch collected from beneath leucaena stands (0–5 cm) being the source in experiment 1 compared with soil from beneath established leucaena (0–15 cm) in the other. Rate of inoculation also differed between experiments, being considerably higher for experiment 1 (90 g/kg soil) than experiment 2 (12.5 g/kg soil).

Calculations based on spore densities of 10–100 propagules/g, normally found in soils suggest that inoculation rates used in experiment 2 (1.3%), were probably inadequate to substantially alter the average propagule density in pots. Although responses to lower inoculation rates have been recorded, this has usually been associated with placement of spores or infected root pieces near root systems of plants being inoculated (Walker and Smith 1984; Haas *et al.* 1986; Ravindra and Bagyaraj 1993).

Although methods are available for producing pure cultures of AM fungi in small quantities, producing them commercially is difficult due to their obligate requirement for a host plant species. In the field, the use of soil as an inoculum is generally considered impractical due to the high volumes of soil necessary to achieve a significant response (Bagyaraj 1994). No response to inoculation with AM fungi was found in leucaena in a field trial at Mt Cotton by Walker (1989) using a soil inoculum added directly to the seed furrows at a rate of 2 kg/5 m of row, equivalent to about 800 kg soil inoculum/ha.

The positive response to inoculation in experiment 1, although still significant 10 weeks after sowing, was mainly due to improved growth before day 56 (data not shown). This was around the time of maximum infection level in uninoculated soil in subsequent experiments. Largest effects of inoculation, therefore, can be expected to occur during early seedling growth in soils where early colonisation is slow.

Phosphorus application

Phosphorus application compensated for slow colonisation of roots by AM fungi in the Mt Cotton soil but only the 1200 kg P/ha rate was able to prevent development of subcritical P concentrations during early growth (Fig. 5*b*). The extreme inefficiency of the leucaena roots uninfected by AM fungi probably makes P application to overcome initial slow colonisation impractical in most circumstances. This is confirmed by the poor growth of leucaena in the fumigated soil even when fertilised at 100 kg P/ha (10% of that in non-fumigated soil).

Results of this and other trials (Brandon and Shelton 1997*a*) have shown that extraction efficiency usually increases with time as roots progressively become infected by AM fungi, making lower rates of application more effective. Long-term responses to large P applications are not expected in soils of moderate fertility. Following colonisation of roots by AM fungi, for example, final dry weight of plants in non-fumigated soil fertilised at 100 kg P/ha was over 90% that of plants fertilised at the very high rate of 1200 kg P/ha. Previous results in the field suggested that long-term limitations due to P occur at soil P levels of 8 mg bicarbonate-extractable P or less, equivalent in this soil to 75 kg P/ha.

One way of increasing early seedling growth in soils where early colonisation is slow may be by banding P near the seed. Results of the current trial would suggest that leucaena is tolerant of high levels of P in the soil. Lu and Miller (1993) found that banding P fertiliser around maize seed significantly increased tissue P concentrations compared with when the same amount of fertiliser was mixed with a larger volume of soil. This approach has not been tested with leucaena.

Grass competition

Grass competition by *Panicum maximum* reduced final plant growth of leucaena by 50% through increased competition for both P and N. Although N concentration increased later in the trial as nodule mass increased, P

concentration remained low at 0.2–0.24% which is close to the critical concentration found by Ruaysoongnern (1990) for inoculated leucaena.

Plotting P content of seedlings against N content indicated that the effects of grass competition were greater on N concentration during early growth, but on P during later growth. This is because, following nodulation, the legume has access to a source of N not available to the grass. Both plants, however, must compete for soil P. The very finely branched root system of the grass may give it an advantage, particularly if colonisation of AM fungi is delayed in the legume.

The extremely poor ability of leucaena seedlings to compete with grasses or weeds has been confirmed in the field (Cooksley 1975; Jones and Aliyu 1976), where in addition to competition for nutrients, competition for water may be significant (Pratchett and Triglone 1990). Careful control of grass and weed competition has been found necessary for successful establishment. In some cases, grass establishment may be best left until the second year (Falvey 1981).

Conclusions

The important role of AM colonisation on early growth, P nutrition, nodulation and N fixation has been confirmed in these experiments. Large effects of AM colonisation on plant growth 41 days after sowing disappeared by the end of the trial (98 days after sowing) due to increased root colonisation. Although the use of soil/mulch as AM fungal inoculum improved seedling growth in 1 experiment, the use of such inoculum is probably impractical in the field due to the high rates of application required. High rates of P fertiliser were needed to compensate for slow early colonisation and would not be economic unless methods can be devised to band the fertiliser around the seed. However, this needs to be further tested in the field.

The much lower rate of infection in the Mt Cotton soil may have been due to long fallow, soil disturbance and periodic waterlogging. Further work needs to be undertaken to evaluate the role of AM fungal colonisation on the early growth of leucaena in a wider range of soils in the field.

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