Effects of arbuscular mycorrhizal fungi on a fallow enriching tree (*Macaranga denticulata*)

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Macaranga denticulata is a fallow enriching species that is important in upland agriculture in parts of northern Thailand. The root zone of this tree supports a high biodiversity of arbuscular mycorrhizal (AM) fungi and it has been postulated that these are important for *Macaranga* to rapidly establish in disturbed fields. To evaluate this, seedlings were inoculated with spores of AM fungi (*Glomus* spp., *G. fasciculatum*, *Acaulospora* spp. and mixed species of AM fungi) collected from the field, and then grown without P fertilizer. Growth of the host was compared with plants grown concurrently at six rates of phosphorus application (0 to150 mg P/kg soil). The experiments were conducted in pots containing 5 kg sterilized soil. Arbuscular mycorrhizal inoculation increased height and plant dry weight. Dry weights of *M. denticulata* inoculated with *Acaulospora* spp. or mixed species of AM fungi were equivalent to uninoculated plants given 150 mg P/kg soil, whereas plants inoculated with *Glomus* spp and *G. fasciculatum* were similar to those with 25 mg P/kg soil. Nutrient contents of plant inoculated with *Acaulospora* spp. and mixed species of AM fungi were higher than plants inoculated with *Glomus* spp and *G. fasciculatum*. Root colonization of plants inoculated with *Acaulospora* spp. and mixed species of AM fungi was not significantly different, and was higher than other inoculated treatments. These experiments have shown that *M. denticulata* is dependent on AM fungi for rapid growth in a low P soil. *Acaulospora morrowiae* appeared to be the dominant species sporulating in root zones of plants inoculated with either *Acaulospora* spp. or mixed species of AM fungi. Further work is required to identify the most effective AM species for *M. denticulata* since the abundance of AM spores in the root zone may not be directly related to the effectiveness of root colonization in nutrient uptake of the tree.

Key words: arbuscular mycorrhizas, phosphorus fertilizer.

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Introduction

Soils of many regions are usually deficient in phosphorus, including the soil in Haui Tee Cha village, Sop Moei district, Mae Hong Son Province (3.0-4.3 mg P/kg soil). Mycorrhizal roots, due to their extramatrical hyphae that are capable of absorbing and translocating nutrients, can explore more soil volume than the non-mycorrhizal roots (Joner and Jakobsen, 1995), and so increase the supply of slowly diffusing ions, such as phosphate to the plant (McArther and Knowles, 1993). Using AM fungi for increasing crop production has recently received considerable attention. Although AM fungi are generally considered to have a broad host ranges, some species are more effective with particular host plants in increasing nutrient uptake and plant growth. Thus, the extent of plant growth promotion by AM fungi can depend upon the specific plant and fungal combination (van der Heijden *et al*., 1998).

Macaranga denticulata Muell. Arg. (*Euphorbiaceae*) is a small to medium-sized, evergreen tree and is a common pioneer species in moist open areas and secondary forests (Kerby *et al*., 2000). In the mountains of Northern Thailand, *M. denticulata* is used as a fallow enriching species by Karen hill tribe farmers (Rerkasem *et al*., 2002). They manage the establishment of the *M. denticulata* canopy in the fallow plots. A very large diversity of AM fungi has been reported to associate with the roots of *M. denticulata* (Youpensuk *et al*., 2004). The objective of this experiment was to examine the effects of different groups of AM fungi (isolated from the root zone of *M. denticulata* in the village of Haui Tee Cha) on growth and nutrient uptake of *M. denticulata* when grown in soil without added P fertilizer, compared with uninoculated plants grown at different levels of P fertilizer.

Materials and methods

Effect of phosphorus application on growth of Macaranga denticulata

The soil used in this experiment had a pH of 5.9 and sandy loam texture (sand 57.5%, silt 24.0% and clay 18.5%). The soil contained 0.1% total N, 4.1 mg/kg available P, 53.0 mg/kg extractable K and 1.9% organic matter. Plastic pots (20.5 cm top diam., 14 cm bottom diam. and 19 cm depth) with a central hole, containing five kg sterilized soil, were used in this trial. Two month-old seedlings of *M. denticulata* growing in sterile soil were used in this experiment with one seedling per pot. Plants were watered once every day with filtered tap water. Every pot received a basal treatment of urea and potassium chloride at the rate of 5 mg N and 5 mg K/kg soil/week, for ten weeks after transplanting.

Triple superphosphate was used as P fertilizer. There were six levels of P: 0, 25, 50, 75, 100 and 150 mg P/kg soil with four replications. The phosphorus fertilizer was applied to the surface of the soil at two weeks after transplanting. At harvest, 3.5 months after transplanting, plant height, shoot and root dry weight were obtained and plant material was analyzed for N (Kjeldahl method), P (Dry ashing and molybdovanado-phosphoric acid method) and K (Dry ashing and atomic absorption spectrophotometer method).

Effect of arbuscular mycorrhizal fungi without phosphorus application on the host plant

The experiment was done in the same soil and concurrently with the above experiment, but P fertilizer was not applied in this experiment. There were five treatments and four replications of AM inoculation: uninoculated control (inoculated with autoclave sieved spores), mixed species sieved spores, spores of *Glomus fasciculatum*, spores of *Glomus* spp., and spores of *Acaulospora* spp. One hundred and fifty AM spores were used in each treatment. Spores were obtained from indigenous soil in the root zone of *M. denticulata* at the village of Haui Tee Cha in the cool season. Spores were placed at a depth of 3 cm and the seedlings transplanted above. At 3.5 months after transplanting, plant height was recorded. Root samples were taken from each pot using two soil cores, 3 cm diameter, from soil surface to the bottom of the pot taken at mid way between the stem and the pot wall. Soil from one soil core was assessed for spore density and the root sample in this soil core was used to determine root colonization. Roots in the other soil core were washed and dried to determine root dry weight in the soil cores. The remaining roots in pots were washed and dried to measure dry weight. After drying, shoots and roots were ground and analyzed for N, P and K contents.

Evaluation of arbuscular mycorrhizal colonization

Roots samples were washed over a 2 mm sieve under running water. The root samples were cut into pieces 1-2 cm in length, cleared in 10% KOH at 121^{o}C for 15 min and rinsed with water on a sieve. Cleared roots were stained with 0.05% trypan blue in lactoglycerol at 121^{o}C for 15 min (Brundrett *et al.*, 1996). Thirty pieces of root fragments were taken at random from each sample and mounted on glass slides to assess AM colonization according to the method of McGonigle *et al*. (1990).

Determination of arbuscular mycorrhizal spore density

Arbuscular mycorrhizal spores were separated from 50 g of each soil sample by wet sieving and 50% sucrose centrifugation (Brundrett *et al*., 1996). Spores were counted under a stereomicroscope.

Statistical analysis

Statistical tests were performed with SPSS for Window version 10. The data were analyzed by analysis of variance (ANOVA) to test the effect of the factors or treatments. Duncan's Multiple Range Test and least significantly difference (LSD) at $P = 0.05$ were used to compare means.

Results and discussion

Effect of phosphorus application on growth of Macaranga denticulata

Height and dry weight of *M. denticulata* increased with increasing P levels. The strongest effect of P on plant height and dry weight was found with the first 25 mg P/kg soil application (Table 1). Plant height and dry weight continued to increase with further increase in the level of P application, but at slower rates. While shoot dry weight and plant height reached their maximum at 100 mg P/kg soil, root dry weight and total dry weight was greatest at 150 mg P/kg soil.

Effect of arbuscular mycorrhizal inoculation on growth of Macaranga denticulata

Growth of *M. denticulata* was greatly enhanced by AM inoculation. The results varied with the different kinds of AM fungi. Shoot dry weight of the host plant in treatments inoculated with *Acaulospora* spp. and mixed species of AM fungi were about seven times greater than the control, and *Glomus fasciculatum* and *Glomus* spp. were about three and four times greater than the control, respectively (Table 2). In the root zone of plants inoculated with mixed species of AM fungi, *Acaulospora* was the dominant genus. This study has shown *Acaulospora* spp. to be about twice as effective in promoting growth of *M. denticulata* than *G. fasciculatum* or *Glomus* spp. Smith and Read (1997) reported that there is increasing evidence of host-specific differences in plant response to AM fungi and in fungal response to plants. Species and strains of AM fungi have been shown to differ in the extent to which they increase plant

Table 1. Effect of phosphorus application on height, shoot and root dry weight of 5.5 month-old *M. denticulata* seedlings.

P applied (mg P/kg)	Height (cm)	Shoot dw. (g)	Root dw. (g)	Root : shoot ratio
0	18.05 c	5.27 b	4.00 d	0.8 : 1
25	32.28 b	12.46 a	7.94 cd	0.6 : 1
50	39.18 ab	16.82 a	10.72 bc	0.6 : 1
75	40.13 ab	17.66 a	11.46 abc	0.7 : 1
100	44.88 a	19.38 a	13.04 ab	0.7 : 1
150	41.63 ab	18.44 a	15.25 a	0.8 : 1
P effect	***	**	***	ns

Means in the same column followed by different letters are significantly different by Duncan's Multiple Range Test. **, ***, significant at P<0.01 and 0.001, respectively; ns, not significant.

Table 2. Effect of AM fungi on height, shoot and root dry weight of 5.5 month-old *M. denticulata* seedlings.

AM fungi	Height (cm)	Shoot dw. (g)	Root dw. (g)	Root : shoot ratio
Uninoculated	16.13 d	3.15 c	2.88 d	0.9 : 1
G. fasciculatum	24.18 cd	9.49 b	5.68 cd	0.6 : 1
Glomus spp.	32.18 bc	13.43 b	9.34 c	0.7 : 1
Acaulospora spp.	46.15 a	20.98 a	14.67 b	0.7 : 1
Mixed species	38.10 ab	23.32 a	19.61 a	0.8 : 1
Inoculation effect	***	***	***	**

Means in the same column followed by different letters are significantly different by Duncan's Multiple Range Test. **, ***, significant at P<0.01, 0.001, respectively.

Table 3. Effect of AM fungal inoculation on root colonization and spore density in the root zones of 5.5 month-old *M. denticulata*.

AM fungi	Root colonization (%)	Spore density (spores/g soil)
Uninoculated	0.0 d	0 e
G. fasciculatum	39.2 c	7 d
Glomus spp.	49.9 b	18 c
Acaulospora spp.	63.0 a	50 a
Mixed species	64.8 a	27 b
Inoculation effect	***	***

Means in the same column followed by different letters are significantly different by Duncan's Multiple Range Test. ***, significant at P<0.001.

growth. For example, Rajan *et al*. (2000) showed that *G. leptotichum* was the best AM symbiont for growth of teak (*Tectona grandis*) from nine species of AM fungi in soil low in available P at pH 5.6. Arbuscular mycorrhizal inoculation had a significant effect on the root to shoot ratio of *M. denticulata*. The highest root to shoot ratio was found in the uninoculated control. Higher root to shoot ratios of uninoculated plants compared to inoculated plants are often found to improve P nutrient acquisition (Marschner, 1991). Thus, phosphorus-deficient plants often allocate more biomass into roots than shoots (Marschner *et al*., 1996). Total dry weight of plant inoculated with *Glomus* spp. and *G. fasciculatum* were about the same as in plants supplied with 25 mg P/kg soil. By contrast, the total dry weight of *M. denticulata* in treatments inoculated with *Acaulospora* spp. was about the same as for plants given 150 mg P/kg soil. Plants inoculated with *Acaulospora* spp and mixed species of AM fungi were not significantly different in total dry weight (Fig. 1).

***Effect of phosphorus application on nutrient contents in* Macaranga denticulata**

Increasing levels of P fertilizer increased N, P and K contents in *M. denticulata*. Whereas N and K contents (Figs. 2 and 4) tended to reflect the accumulation of dry matter (Fig. 1), the P content increased linearly with P supply up to 150 mg P/kg soil (Fig. 3).

***Effect of arbuscular mycorrhizal inoculation on nutrient contents of* Macaranga denticulata**

The greatest N, P and K contents occurred in plant inoculated with *Acaulospora* spp. and mixed species of AM fungi (Figs. 2, 3 and 4). Nitrogen and P contents of plants inoculated with *Glomus* spp. and *G. fasciculatum* were not significantly different, but K content in plants inoculated with *Glomus* spp. was significantly higher than in the *G. fasciculatum* plants. Abbott and Robson (1984) reported that all AM fungi do not contribute equally to nutrient uptake and plant growth. Jakobsen *et al*. (1992) studied hyphal transport of ^{32}P by three AM fungi (*A. laevis*, *Glomus* sp. and *Scutellospora calospora*) associated with *Trifolium subterraneum*. The result showed that *A. laevis* transported ^{32}P over longer soil-root distances than *Glomus* sp. or *S. calospora*. This was due to the ability of *A. laevis* to spread hyphae faster in soil than the other two species. In this study, plants inoculated with *G. fasciculatum* and *Glomus* spp. had N contents similar to plants supplied with 25 and 50 mg P/kg soil, respectively. Nitrogen contents of plants inoculated with *Acaulospora* spp. and mixed species of AM fungi were not significantly different from plants given 150 mg P/kg soil (Fig. 2). Plants inoculated with *G. fasciculatum* and *Glomus* spp. had P contents about the same as plants supplied with 25 and 50 mg P/kg soil, respectively, while plants inoculated with *Acaulospora* spp. and mixed species of AM fungi were not significantly different from plants given 75 mg P/kg soil (Fig. 3). Potassium accumulation in *M. denticulata* inoculated with *G.*

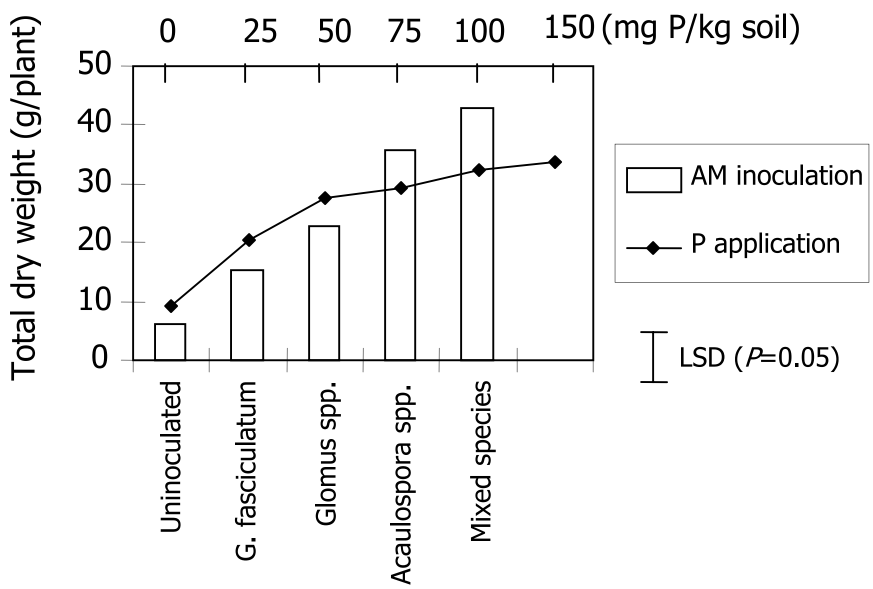

Fig. 1. Comparison of total dry weight of *M. denticulata* from the effects of AM fungal inoculation and phosphorus application.

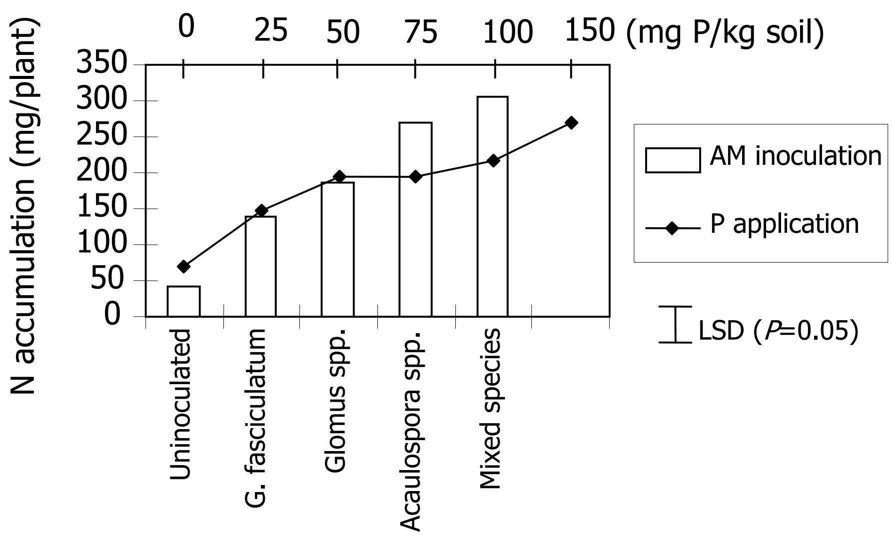

Fig. 2. Comparison of the effects of AM fungal inoculation and P application on nitrogen accumulation in *M. denticulata*.

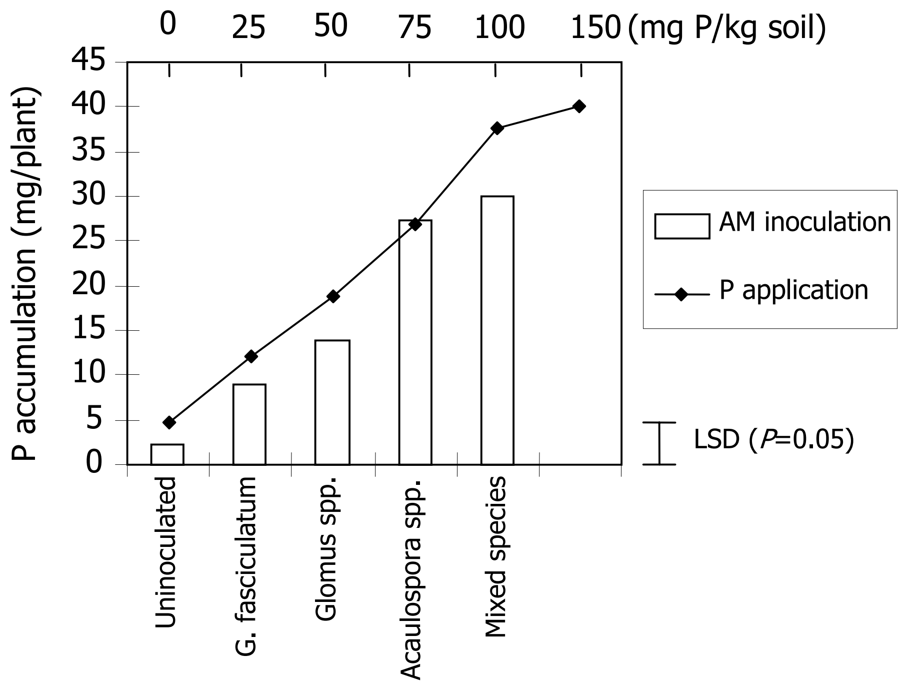

Fig. 3. Comparison of the effects of AM fungal inoculation and P application on phosphorus accumulation in *M. denticulata*.

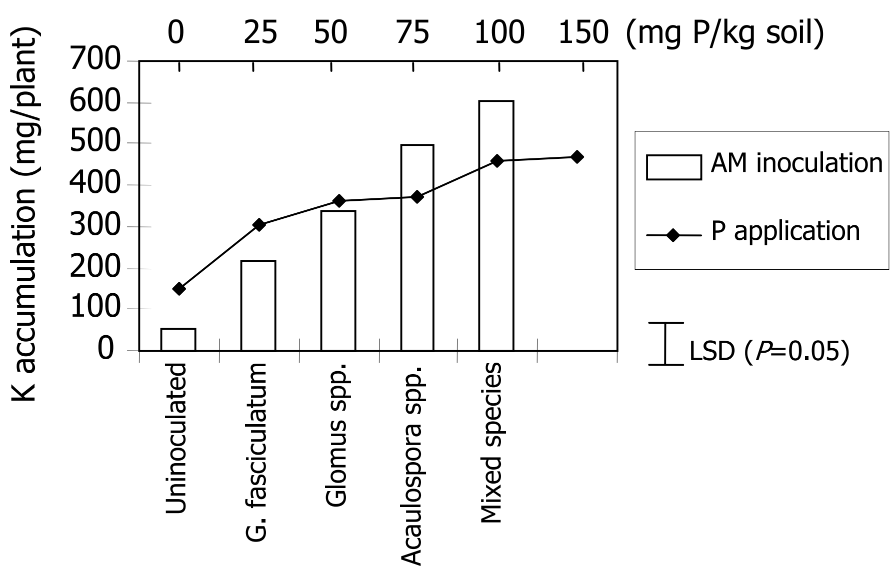

Fig. 4. Comparison of the effects of AM fungal inoculation and P application on potassium accumulation in *M. denticulata*.

fasciculatum did not differ from plants supplied with 25 mg P/kg soil. Plants inoculated with *Glomus* spp. had total K contents similar to plants given 50 mg P/kg soil. Plants inoculated with *Acaulospora* had similar K contents to uninoculated plants given 150 mg P/kg soil. Inoculation with mixed species of AM fungi had the greatest effect on K accumulation in *M*. *denticulata* (Fig. 4). From the effect of P application, it is clear that P is the limiting factor in this soil. Therefore, the enhancement of P uptake by AM fungi overcame P deficiency by increasing the uptake of this nutrient from the soil. The increase in N and K contents of inoculated plants may be a result of the removal of P deficiency by AM fungi. However, some N and K uptake may be also enhanced by AM fungi. Some researchers have reported that AM hyphae can transfer nutrients other than P such as N, K and Zn to the host plant (Frey and Schuepp, 1993; Marscher and Dell, 1994; Zhu *et al*., 2000).

Effect of arbuscular mycorrhizal inoculation on root colonization and spore density in the root zone of **Macaranga denticulata**

Uninoculated controls plants were non mycorrhizal. Root colonization ranged in the inoculated treatments from 39.2 to 64.8%. Root colonization of treatments inoculated with *Acaulospora* spp. and mixed species of AM fungi were not significantly different. Root colonization and spore density in the root zone of *M*. *denticulata* inoculated with *Acaulospora* spp. and mixed species of AM fungi were significantly higher than other treatments (Table 3). Spores of *Acaulospora morrowiae* were the dominant species found in the root zones of plants inoculated with either *Acaulospora* spp. or mixed species of AM fungi, indicating the better sporulating ability of this fungus with *M*. *denticulata* as the host plant in this soil. Bever (2002) found that AM fungal species, though associating with all hosts, have host-specific differences in their population growth rates. He suggested that the relative growth rates of AM fungal species depends not only on the identity of the host with which they are associated, but also on the identity of other components of the AM fungal community or other components of the soil community. Abbott and Gazey (1994) reported that fungi differ in the manner and extent to which they colonize roots. They also differ in their capacity to form propagules. In this study, growth and nutrient contents of the host plants were positively related to percentage of root colonization. Many studies (Ahiabor and Hirata, 1994; Clark and Zeto, 1996; Clark *et al*., 1999; Bagayoko *et al*., 2000) have shown that the extent of the response of the host plant varies with species of AM fungi, and that a high percentage of root colonization generally enhanced shoot dry weight and nutrient uptake of the host plant.

This study has demonstrated that *M. denticulata* plants respond well to AM inoculation under pot conditions. The pot trial suggests that AM fungi may play an important role in the rapid growth and nutrient uptake of *M. denticulata*, a fallow enriching tree, especially on soils that are low in available phosphorus. Furthermore, significant variation in the effectiveness of different AM species was demonstrated, with *Acaulospora* spp. and mixed species of AM fungi stimulating maximum growth and nutrient uptake in a low P soil. Further work will investigate the effectiveness of single AM fungal species for growth of *M. denticulata*.

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