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Effects of Arbuscular Mycorrhizal Inoculation and Fertilizer on Production of *Castanopsis acuminatissima* Saplings for Forest Restoration in Northern Thailand

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Abstract: *Castanopsis acuminatissima* is a native tree used to restore forest in Thailand. To accelerate seedling growth experiments were carried out to determine the efficacy of applying to *C. acuminatissima*. Arbuscular Mycorrhizal (AM) fungi, produced on sorghum, were used as inoculum to investigate the symbiosis on seedlings. The effects of AM inoculation (*Acaulospora elegans*, *Glomus etunicatum*, *Glomus mosseae*) together with phosphate fertilization (KH_2PO_4) on seedlings in a P-deficient soil were studied under greenhouse conditions. Increasing P-application rates greatly enhanced seedling growth (maximum at 250 mg kg^{-1} soil). Growth was most rapid with *G. etunicatum*-colonized plants with P application (40.8 cm), whereas much lower height was found with non-AM plants without P added (14.4 cm). The mycorrhizal effective for *C. acuminatissima* in previous experiments were confirmed by growing seedlings in a forest soil with slow-release fertilizer (NPK) and combined with AM species under nursery performance conditions. Plant height was significantly enhanced by fertilizer but not by fungi. The greatest height was found in non-AM plants with fertilization (14.5 cm), whereas lower height was found for non-AM plants with no fertilizer added (10.9 cm). AM inoculation greatly enhanced seedling growth in P-deficient soil more than in forest soil due to differences in abilities of AM species to establish a symbiosis. Therefore, in sapling production, the soil properties and level of fertilization should be evaluated keeping secondary effects caused by changed mycorrhizal association.

Key words: Arbuscular mycorrhizal fungi, framework tree species, forest restoration, mycorrhizal seedling production, phosphorus fertilizer

INTRODUCTION

Forest restoration means the re-establishment of the original forest ecosystem that was present before deforestation occurred. The goals of forest restoration are environmental protection and wildlife conservation (Anonymous, 2006). In 1994, the Forest Restoration Research Unit at Chiang Mai University (FORRU), started to investigate the possibility of restoring forests on degraded sites in northern Thailand by adapting the framework species method (first developed in Queensland, Australia) (Goosem and Tucker, 1995; Anonymous, 2006) to local conditions. FORRU screened indigenous forest tree species to select potential candidate framework species for field trials. In the FORRU's research tree nursery, experiments were designed to develop seedling production for high quality planting stock. *Castanopsis acuminatissima* (Bl.) A. DC. (Fagaceae), was confirmed as a potential framework species that could be used to restore seasonally dry tropical forest in northern Thailand but this species grow relatively slowly and difficult to raise in nursery. To solve such problems, studies have been made on modified potting media and fertilizer application (Anonymous, 2006). Arbuscular Mycorrhizal (AM) fungi have form symbiosis with a wide range of forest tree

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species (Gai *et al*., 2006). Such symbioses provide many benefits to host trees and are especially important in development of seedlings grown in nurseries and establishment of saplings planted in deforested sites.

AM symbioses result in increased growth of plants depending on the fungal strains (Pattinson *et al*., 2004; Youpensuk *et al*., 2005). AM fungi also protect plants against root pathogens, confer resistance to drought and increase soil aggregation (Dubský *et al*., 2002; Rilling *et al*., 2005; Wu *et al*., 2006). Lack of nutrient availability in tropical soils often limits plant growth. The ability of AM fungi to enhance nutrient absorption, (particularly phosphorus) by hyphal uptake and translocation towards the plant, is an important advantage (Koide and Mosse, 2004). The possibility of using beneficial attributes of AM fungi in planting stock will depend on preliminary assessments of whether inoculation is a suitable management option. AM fungi are obligate symbionts, usually propagated by growing them with living host plants in pot cultures. For starting pot cultures of AM fungi, the combination of appropriate host plant and substrate media for production of mycorrhizal inoculum is crucial (Setiadi, 2000). Pot cultures, which consist of soil, spores and mycorrhizal roots etc., can be used as inoculum for experiments or applied to seedling grown in a nursery or broadcast in the field (Brundrett *et al*., 1996; Setiadi, 2000; Klironomos and Hart, 2002). Knowledge about the ability of plant species to form symbiosis with AM fungi is very important for restoration success and indicates the need for inoculum in plants cultivated in forest nurseries (Wubet *et al*., 2003). The purposes of present experiment were to: (1) select appropriate host plants under pot culture conditions for production of indigenous AM fungal inoculum in the nursery, (2) examine the effects of 3 AM species with 6 rates of P application on plant development in P-deficient soil medium under greenhouse conditions and (3) examine the effects of AM fungal inoculation and conventional fertilization on growth of seedlings in forest soil under nursery performance conditions.

MATERIALS AND METHODS

Inoculum Production

The first experiment consisted of 30 treatments with 6 indigenous AM species [*Acaulospora elegans* Trappe and Gerd., *A. mellea* Spain and Schenck, *A. scrobiculata* Trappe, *Glomus etunicatum* Becker and Gerd, *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe and *Scutellospora heterogama* Walker and Sanders] and 5 host plants [maize (*Zea mays* L.), marigold (*Tegetes erecta* L.), soybean (*Glycine max* (L.) Merr.), sorghum (*Sorghum vulgare* Pers.) and upland rice (*Oryza sativa* L. cv. Bue Bang)] with 3 replications. The experiment was undertaken in clay pots (22 cm top diameter, 18 cm bottom diameter and 19 cm depth) with drainage hole containing 3 kg P-deficient soil medium (P-deficient soil and coarse sand ratio 2:1), autoclaved twice at 121°C for 30 min with a 2 day interval. The soil pH (H_2O) was 5.98 and contained 0.041% total N (Kjeldahl method), 1.4 mg kg^{-1} available P (Bray II method) and 44.0 mg kg^{-1} extractable K (1 M NH_4OAc, pH 7). Seeds were surface-sterilized with 10% sodium hypochlorite for 5 min, rinsed with sterile water and sown in Petri dishes, containing the moist tissue paper for 1 week. Seedlings were transplanted 3 seedlings per pot. AM spores were extracted from the soil samples by wet-sieving and 50% sucrose centrifugation (Brundrett *et al*., 1996) and collected on a 53 μm sieve. Fifty spores were inoculated into each pot. Seedlings were grown in a greenhouse at the Chiang Mai University (CMU) for 4 months between November 2005 and February 2006. Seedlings were watered once every 2 days with 500 mL of tap water. Twice a month, 80 mL of 4 full strength Hoagland' solution (Hoagland and Arnon, 1950), without P was added to each pot. At harvest, root samples were separated from soil and cleaned with tap water. The root samples were cleared in 10% KOH at 121°C for 15 min and stained with 0.05% trypan blue in lactoglycerol (Brundrett *et al*., 1996). Thirty stained root segments from each plant (1 cm long) were taken at random and mounted on microscopic slides to assess mycorrhizal colonization (McGonigle *et al*., 1990). One hundred gram dried soil of all different treatments were used to determine spore density.

Effects of AM fungi with Phosphorus Fertilizer on Seedling Growth in Greenhouse Experiment

The second experiment consisted of 90 pots with 5 inoculation treatments (no inoculation and AM inoculation: *A. elegans*, *G. etunicatum*, *G. mosseae* and mixed AM species) and 6 levels of P application (KH_2PO_4) (at the rates of 0, 50, 100, 150, 200 and 250 mg P kg^{-1} medium) with 3 replications. Spores of AM species were produced on sorghum pot cultures in P-deficient soil medium as shown above and used for inoculation. The experiment was undertaken in clay pots containing 3 kg autoclaved P-deficient soil medium. Seeds of *C. acuminatissima* were surface-sterilized with 10% sodium hypochlorite for 10 min, rinsed with sterile water and sown in a plastic tray containing the autoclaved forest soil medium (primary evergreen forest soil, coconut husk and peanut husk ratio 2:1:1). The medium pH (H_2O) was 5.60 and contained 0.628% total N, 15.8 mg kg^{-1} available P and 132.0 mg kg^{-} extractable K. Three month-old seedlings (5-6 cm tall) were transplanted one seedling per pot. In each AM treatment, 150 spores were inoculated into each pot. Seedlings were grown in a greenhouse at the CMU for 6 months between December 2005 and March 2006. Seedlings were watered once every 2 days with 500 mL of tap water. Two weeks after transplanting, 6 levels of KH_2PO_4 were added to each treatment. Twice a month, 80 mL of 4 full strength Hoagland' solution without P was added to each pot. At harvest, height and stem diameter of seedlings were measured. Roots were divided into 2 random sub-samples. Shoot samples and one root sub-sample were oven dried at 60°C for 48 h. Dry samples were analyzed for P content by the dry ashing and molybdovanado-phosphoric acid method. The second root sub-sample was used to determine AM colonization and soil sub-samples were assessed for spore density.

Effects of AM fungi with Slow-Release Fertilizer on Seedling Growth in Nursery Sapling Production

The third experiment consisted of 10 treatments with 5 inoculation treatments (no inoculation and AM inoculation: *A. elegans*, *G. etunicatum*, *G. mosseae* and mixed AM species) and 2 levels of slow-release fertilizer (NPK 14-14-14) (at the rates of 0 and 375 mg kg^{-} medium) with 28 replications. Slow-release fertilizer has been used successfully at FORRU for many framework species (Anonymous, 2006). The experiment was undertaken in plastic bags (23×6 cm) with drainage holes, containing 800 g autoclaved forest soil medium. Three month-old seedlings of *C. acuminatissima* were transplanted one seedling per bag. In each AM treatment, 150 spores were inoculated into each pot. Slow-release fertilizer was applied in fertilization treatments at the start of the experimental period. Seedlings were grown at the FORRU's nursery for 6 months between April and September 2006. Seedlings were watered once a day with tap water. Every 3 months, slow-release fertilizer was applied to each treatment. At harvest, height and stem diameter of seedlings were measured. Fresh shoot and root samples were treated in the same way as described previously. Dry plant samples were analyzed for P content. Root sub-samples were used to determine colonization percentage and soil sub-samples were assessed for spore density.

Data Analysis

All data were subjected to analysis of variance (ANOVA) for a completely randomized design. Residuals were normally distributed with constant variance. SPSS software version 12.0 was used to conduct the ANOVA. Duncan's Multiple Range Test ($p<0.05$) was used to compare treatment means.

RESULTS

Inoculum Production of Arbuscular Mycorrhizal Fungi

Four months after starting the pot culture, all 5 host plant species inoculated with 6 AM species had developed mycorrhizas. Spore density and colonization percentage varied greatly among the

Table 1: Spore density of AM fungi (per 100 g dry wt. soil) (n = 3) in pot cultures with 5 host plants inoculated with spores of 6 AM fungal isolates

	Spore density of AM fungi (spores/100 g soil)					
Host plant	*A. elegans*	*A. mellea*	*A. scrobiculata*	*G. etunicatum*	*G. mosseae*	*S. heterogama*
G. max	426.33dB	13.33 nsC	14.67abC	598.00cdA	40.00dC	9.33bC
O. sativa	1683.33bcA	14.67 nsD	13.33abD	396.00dB	190.00bcC	6.67bD
S. vulgare	2713.33aB	13.33 nsC	16.67abC	6148.67aA	642.67aC	22.67aC
T. erecta	967.67cdB	9.33 nsC	6.00bC	1457.33cA	102.00cdC	11.33abC
Z. mays	1867.33bB	10.00 nsC	19.33aC	3933.33bA	231.33bC	4.67bC
Analysis of variance						
AM species			***			
Host plant species			***			
AM species × Host plant species			***			

Means followed by the same letter (s) (lower case within columns and capitals within rows) are not significantly different by Duncan's Multiple Range Test; ns: not significant. ***: significant at p<0.001

Table 2: Root colonization (per 30 root pieces of plant species) (n=3) in pot cultures with 5 host plants inoculated with spores of 6 AM fungal isolates

	AM colonization (%)					
Host plant	*A. elegans*	*A. mellea*	*A. scrobiculata*	*G. etunicatum*	*G. mosseae*	*S. heterogama*
G. max	87.95aB	20.00nsC	14.81abC	90.00bB	100.00aA	5.00cD
O. sativa	73.00bB	11.11nsC	3.70abC	100.00aA	95.00bA	3.33cC
S. vulgare	100.00aA	51.85nsC	13.33abD	100.00aA	100.00aA	78.33aB
T. erecta	92.16aAB	73.33nsB	16.66bC	93.33abAB	100.00aA	25.00bC
Z. mays	93.79aA	55.00nsB	56.67aB	100.00aA	100.00aA	11.66cC
Analysis of variance						
AM species			***			
Host plant species			***			
AM species × Host plant species			***			

Means followed by the same letter (s) (lower case within columns and capitals within rows) are not significantly different by Duncan,s Multiple Range Test; ns: not significant. ***: significant at p<0.001

different host species. Spore density and mycorrhizal colonization were increased by the host species and AM species which interacted (Table 1 and 2). Three fungal species: *A. elegans*, *G. etunicatum* and *G. mosseae* produced significantly the highest spore densities and colonization abilities on *S. vulgare* and *Z. mays*. The spore density of these AM species on sorghum was significantly higher than on maize, whereas mycorrhizal colonization on both plants did not differ from each other (93.8-100.0%). The highest spore number were found on sorghum inoculated with *G. etunicatum* (6148.7 spores/100 g soil), *A. elegans* (2713.3 spores/100 g soil) and *G. mosseae* (642.7 spores/100 g soil), respectively. Whilst much lower densities and root colonization of other 3 AM species were found on all host species (Table 1 and 2).

Effects of AM Inoculation with P Application on Growth of *C. acuminatissima* in P-Deficient Soil Medium

Growth of *C. acuminatissima* seedlings in the P-deficient soil experiment was highly influenced by both AM inoculation and P-application rates (Table 3). Six months after transplant, plant height, shoot and root dry weights as well as shoot P content were all significantly increased by both factors which interacted, whereas root to shoot ratio and root P content were also increased by both factors but not by their interaction. The only exception was stem diameter, which was only increased by P rates. AM colonization was only increased by fungal inoculation, whereas spore density on the other hand, was increased by both P rates and fungus which interacted (Table 3). Root colonization ranged in the P applied treatments from 36.7-45.4%, which did not differ with P rates (Table 3). In the AM treatments, colonization percentages ranged from 40.1-55.3%. Root colonization in the plants

Table 3: Effects of AM inoculation and P application (KH_2PO_4) on growth of *C. acuminatissima* seedlings grown in P-deficient soil medium and root colonization and spore density of AM fungi in plant rhizoshere (n = 3)

Treatments	Plant height (cm)	Stem diameter (cm)	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})
AM inoculation				
Uninoculation	17.89c	0.23ns	0.66c	0.89d
A. elegans	24.25b	0.24ns	1.40ab	2.59a
G. etunicatum	27.36a	0.23ns	1.60a	2.08bc
G. mosseae	22.44b	0.24ns	1.17b	2.04c
Mixed species	19.85c	0.22ns	0.84c	2.04a
P applied (mg P kg^{-1}medium)				
0	15.29d	0.21c	0.54c	1.36c
50	16.30d	0.21c	0.58c	1.51c
100	19.87c	0.22bc	0.77c	1.67c
150	25.80b	0.23bc	1.49b	2.33b
200	32.11a	0.24b	1.36b	2.39b
250	17.86a	0.27a	2.07a	2.77a
Analysis of variance				
AM inoculation	***	ns	***	***
P applied	***	***	***	***
AM inoculation × P applied	***	ns	**	*

Treatments	Root to shoot ratio (dry weight)	Shoot P content (mg plant^{-1})	Root P content (mg plant^{-1})	Root colonization (%)	Spore density (spores/100g soil)
AM inoculation					
Uninoculation	1.44c	0.20c	0.22d	0.00c	0.00d
A. elegans	2.26b	0.64a	0.76a	53.72a	23.11c
G. etunicatum	1.86bc	0.74a	0.67ab	55.33a	31.83b
G. mosseae	2.28b	0.42b	0.50bc	55.26a	33.50ab
Mixed species	2.83a	0.40b	0.45c	40.10b	38.33a
P applied (mg P kg^{-1}medium)					
0	2.68a	0.15c	0.25c	43.92ns	24.20abc
50	2.73a	0.18c	0.29c	45.39ns	28.07a
100	2.37ab	0.23c	0.32c	38.14ns	27.00ab
150	1.81bc	0.61b	0.62b	41.47ns	30.07a
200	1.89bc	0.52b	0.63b	39.64ns	21.93bc
250	1.35c	1.21a	1.02a	36.71ns	20.87c
Analysis of variance					
AM inoculation	***	***	***	***	***
P applied	**	***	***	ns	**
AM inoculation × P applied	ns	***	0.074	ns	*

Means in the same column followed by different letter(s) are significantly different by ANOVA and Duncan, s Multiple Range test. *, **, ***: Significant at $p<0.05$, 0.01, 0.001, respectively; ns: not significant

inoculated with the single species of AM fungi was significantly higher than for the mixed species inoculum. Spore density was significantly increased by P rates and AM fungi which interacted. Spore density in the P applied treatments ranged from 20.9-30.1 spores/100 g soil which reached maximum at 150 mg kg^{-1}soil (30.1 spores/100 g soil) and continued to decrease with the lowest at 250 mg kg^{-} soil (20.9 spores/100 g soil). Spore density ranged from 23.1-38.3 spores/100 g soil and the highest density was found in plants inoculated with mixed AM species.

P applications were highly beneficial for growth parameters of plants as measure by height, stem diameter (Table 4), dry weights (Table 5) and P contents (Table 6) and AM inoculations also had significant effects on plant growth. Six months after transplant, non-AM plants grown in P-deficient soil exhibited increasing either height or shoot dry weight to increasing P rates, whereas the other growth parameters showed no such differences. Contrast with all growth parameters of AM plants, tended to increase with increasing P rates (maximum at 250 mg P kg^{-1}) and mycorrhizal enhancement varied with the different kinds of AM species. In P applied treatments, seedlings generally grew very little and no significant differences between AM plants and non-AM plants were observed for plant height, stem diameter and P contents, whereas dry weights of AM plants significantly trended to be higher than non-AM plants.

Table 4: Height and diameter (n = 3) of *C. acuminatissima* seedlings grown in P-deficient soil medium containing increasing P application with AM inoculation

	Plant height (cm)				
Treatments	Uninoculation	*A. elegans*	*G. etunicatum*	*G. mosseae*	Mixed species
P applied (mg P kg^{-1}medium)					
0	14.44bNS	16.56cNS	15.59bNS	14.84cNS	15.02cNS
50	16.19bNS	18.02cNS	15.97bNS	16.05cNS	15.26cNS
100	18.13bNS	22.12bcNS	18.56bNS	22.19bNS	18.35bcNS
150	17.76bB	27.05bB	39.19aA	25.69bB	19.28bcB
200	17.89bC	26.81bAB	33.97acA	24.03bB	21.21bBC
250	22.92aC	34.95aAB	40.85aA	31.81aABC	30.00aBC
	Stem diameter (cm)				
Treatments	Uninoculation	*A. elegans*	*G. etunicatum*	*G. mosseae*	Mixed species
P applied (mg P kg^{-1}medium)					
0	0.22nsNS	0.21bNS	0.22nsNS	0.19bNS	0.21nsNS
50	0.22nsNS	0.21bNS	0.219nsNS	0.21bNS	0.21nsNS
100	0.22nsNS	0.20bNS	0.23nsNS	0.24abNS	0.22nsNS
150	0.22nsNS	0.24bNS	0.25nsNS	0.25abNS	0.20nsNS
200	0.23nsNS	0.23bNS	0.25nsNS	0.24abNS	0.25nsNS
250	0.24nsB	0.32aA	0.24nsB	0.29aA	0.24nsB

Means followed by the same letter (lower case within columns and capitals within rows) are not significantly different by Duncan's Multiple Range Test; ns: not significant

Table 5: Shoot and root dry weights (n = 3) of *C. acuminatissima* seedlings grown in P-deficient soil medium containing increasing P application with AM inoculation

	Shoot dry weight (g plant^{-1})				
Treatments	Uninoculation	*A. elegans*	*G. etunicatum*	*G. mosseae*	Mixed species
P applied (mg kg^{-1} KH_2PO_4)					
0	0.45bB	0.76bA	0.67bAB	0.42dB	0.42cB
50	0.54bAB	0.83bA	0.51bAB	0.53cdAB	0.46bcB
100	0.65bNS	0.82bNS	0.91bNS	0.96cNS	0.51bcNS
150	0.62bB	1.81abAB	2.63aA	1.45bAB	0.96bB
200	0.62bD	1.64abAB	2.136aA	1.46bBC	0.94bCD
250	1.07aC	2.58aAB	2.74aA	2.18aAB	1.77aBC
	Root dry weigh (g plant^{-1})				
Treatments	Uninoculation	*A. elegans*	*G. etunicatum*	*G. mosseae*	Mixed species
P applied (mg kg^{-1}KH_2PO_4)					
0	0.96nsB	1.77nsA	1.54bAB	1.48cAB	1.05cB
50	0.78nsC	2.28nsA	1.63bB	1.53cB	1.35bcB
100	0.91nsB	1.93nsA	1.42bAB	2.15bA	1.96bA
150	0.76nsC	3.13nsAB	3.51aA	2.34bAB	1.89bBC
200	0.82nsC	3.05nsA	3.11aA	2.13bB	2.83aAB
250	1.11nsB	3.37nsA	3.39aA	2.87aA	3.13aA

Means followed by the same letter (lower case within columns and capitals within rows) are not significantly different by Duncan's Multiple Range Test; ns: Not significant

AM inoculation significantly increased plant height over non-inoculated controls were found in *A. elegans* at 200-250 mg P kg^{-1}, *G. etunicatum* at 150-250 mg P kg^{-} and *G. mosseae* at 200 mg P kg^{-1}. The maximum height was 1.52 and 1.78 fold higher than the control in *A. elegans* and *G. etunicatum* at 250 mg P kg^{-1}, respectively, whereas, height of *G. etunicatum* plants was the highest (40.8 cm) (Table 4). Stem diameter was only influenced by P rates. Stem diameter had a 1.45 and 1.32 fold increase over control with no P added in *A. elegans* and *G. mosseae* at 250 mg P kg^{-1}, respectively, which did not differ from one another (0.3 cm) (Table 4).

Only *A. elegans* plants at the lowest P rate significantly had a higher shoot dry weight than non-AM plants and other AM plants. Whilst at high P rates, AM inoculation significantly increased over

Table 6: Shoot and root P contents (n = 3) of *C. acuminatissima* seedlings grown in P-deficient soil medium containing increasing P application with AM inoculation

	Shoot P content (mg plant^{-1})				
Treatments	Uninoculation	*A. elegans*	*G. etunicatum*	*G. mosseae*	Mixed species
P applied (mg kg^{-1} KH_2PO_4)					
0	0.12nsNS	0.21cNS	0.15bNS	0.11bNS	0.14cNS
50	0.13nsB	0.25cA	0.19bAB	0.15bAB	0.16cAB
100	0.20nsNS	0.20cNS	0.33bNS	0.24bNS	0.17cNS
150	0.19nsB	0.76bAB	1.23aA	0.31bB	0.57bB
200	0.16nsC	0.71bB	1.02aA	0.30bC	0.42bBC
250	0.40nsB	1.70aA	1.55aA	1.44aA	0.96aAB
	Root P content (mg plant^{-1})				
Treatments	Uninoculation	*A. elegans*	*G. etunicatum*	*G. mosseae*	Mixed species
P applied (mg kg^{-1} KH_2PO_4)					
0	0.21nsNS	0.29bNS	0.30bNS	0.23bNS	0.20dNS
50	0.21nsC	0.40bA	0.35bAB	0.26bBC	0.21dC
100	0.22nsNS	0.34bNS	0.35bNS	0.38bNS	0.31cdNS
150	0.23nsB	0.94abA	0.98abA	0.54bAB	0.43cAB
200	0.20nsB	1.05abA	0.85abAB	0.40bAB	0.64bAB
250	0.26nsB	1.54aA	1.18aA	1.22aA	0.89aAB

Means followed by the same letter (s) (lower case within columns and capitals within rows) are not significantly different by Duncan's Multiple Range Test; ns: not significant

non-AM plants were found in most fungus treatments, except for mixed fungal species. At 250 mg P kg^{-1}, the maximal shoot biomass exhibited 2.41, 2.56 and 2.34 fold increase over control in *A. elegans*, *G. etunicatum* and *G. mosseae*, respectively, whereas *G. etunicatum* plants gave the highest shoot biomass (2.7 g plant^{-1}) (Table 5). *A. elegans* plants with no P added also had a significantly higher root dry weight than non-AM plants and other AM plants. AM inoculation significantly increased over non-AM plants were found in all fungus treatments at the first 50 mg P kg^{-1} and continued to increase with further increase in the level of P rates. Root biomass reached their maximum at 250 mg P kg^{-1} and exhibited 3.04, 3.05, 2.58 and 2.82 fold increase over non-inoculated controls in *A. elegans* and *G. etunicatum G. mosseae* and mixed AM species, respectively, which did not differ from one another (2.8-3.4 g plant^{-1}) (Table 5).

At 50 mg P kg^{-1}, AM inoculation significantly increased shoot P content over non-AM plants was only found in *A. elegans*. Whilst at higher P rates, AM inoculation significantly increased shoot P content were found in *A. elegans* at 200-250 mg P kg^{-1}, *G. etunicatum* at 150-250 mg P kg^{-} and *G. mosseae* at 250 mg P kg^{-1}. At the highest rate, the maximal shoot P content exhibited 3.04, 3.05, 2.58 and 2.82 fold increase over non-AM plants in *A. elegans* and *G. etunicatum* and *G. mosseae*, respectively which did not differ from one another (1.4-1.7 mg plant^{-1}) (Table 6). Fungal inoculation significantly increased root P content over non-inoculated controls were found in *A. elegans* and *G. etunicatum* at first 50 mg P kg^{-1} and still increased at 150 mg P kg^{-1}. Whilst at 200 mg P kg^{-1}, only root P content of *A. elegans* plants were significant increased over controls. At 250 mg P kg^{-1}, root P content of most AM plants exhibited 5.92, 4.54 and 4.69 fold increase over non-AM plants in *A. elegans*, *G. etunicatum* and *G. mosseae*, respectively which did not differ from one another (1.2-1.5 mg plant^{-1}) (Table 6).

Effects of AM Inoculation with Slow-Release Fertilizer on Growth of *C. acuminatissima* in Forest Soil Medium

Growth of *C. acuminatissima* seedlings in the forest soil measured for 6 months showed consistent effects of slow-release fertilization throughout the experiment (Table 7). Plant height, dry weights and P contents were increased by fertilizer but not by AM fungi. However, fertilization effects

Table 7: Effects of AM inoculation and slow-release fertilizer application on growth of *C. acuminatissima* seedlings grown in forest soil medium and root colonization and spore density of AM fungi in plant rhizoshere (n = 28)

Treatments	Plant height (cm)	Stem diameter (cm)	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})
Uninoculated	10.89cd	0.15cd	0.39bcd	0.62bcd
Uninoculated+Fertilizer	14.54a	0.16bc	0.58ab	0.78ab
A. elegans	10.89cd	0.15bcd	0.25d	0.51d
A. elegans+Fertilizer	11.41bcd	0.16bc	0.62a	0.61bcd
G. etunicatum	9.71d	0.14d	0.43abcd	0.64bcd
G. etunicatum+Fertilizer	12.73abc	0.17b	0.42abcd	0.76ab
G. mosseae	10.04d	0.15bcd	0.35cd	0.57cd
G. mosseae+Fertilizer	13.13ab	0.16bc	0.62a	0.83a
Mixed species	11.52bcd	0.16bc	0.39bcd	0.63bcd
Mixed species+Fertilizer	13.05ab	0.20a	0.52abc	0.70abc
Analysis of variance				
AM inoculation	0.075	*	ns	0.061
Fertilization	***	***	***	***
AM inoculation × Fertilization	ns	ns	0.053	ns

Treatments	Root to shoot ratio (dry weight)	Shoot P content (mg plant^{-1})	Root P content (mg plant^{-1})	Root colonization (%)	Spore density (spores 100 g^{-1} soil)
Uninoculated	1.83bc	0.05cd	0.04c	0.00f	0.00f
Uninoculated+Fertilizer	1.77cd	0.13b	0.10a	0.00f	0.00f
A. elegans	2.58a	0.03d	0.03c	52.56d	22.43e
A. elegans+Fertilizer	1.14d	0.19a	0.10a	79.52a	57.71ab
G. etunicatum	2.44ab	0.04d	0.03c	41.96e	20.86e
G. etunicatum+Fertilizer	1.95bc	0.10bc	0.09ab	65.30c	16.14e
G. mosseae	1.72cd	0.04d	0.03c	38.38e	42.28d
G. mosseae+Fertilizer	1.89bc	0.15ab	0.12a	77.58ab	49.14cd
Mixed species	1.65cd	0.07cd	0.05bc	58.87cd	65.14ab
Mixed species+Fertilizer	1.77cd	0.16ab	0.12a	68.32bc	74.57a
Analysis of variance					
AM inoculation	ns	ns	ns	***	***
Fertilization	*	***	***	***	***
AM inoculation × Fertilization	***	ns	ns	***	***

Means in the same column followed by different letter (s) are significantly different by ANOVA and Duncan, s Multiple Range Test. * , **, ***: Significant at $p<0.05$, 0.01, 0.001 respectively; ns: Not significant

on growth parameters were slightly higher it increased growth than non-AM plants without fertilization (controls). Plant growth was highest in all fertilization treatments for plant height (14.5 cm), shoot and root dry weights (0.6 and 0.8 g plant^{-1} , respectively) and shoot and root P contents (0.2 and 0.1 mg plant^{-1} , respectively). Whilst significant mycorrhizal effects were only found in stem diameter, which fungal-fertilizer interactions were not found (Table 7). Stem diameter of *G. etunicatum* and mixed AM species plants with fertilization were highly increased by AM fungi, whereas the highest diameter was found in AM plants with fertilization (0.2 cm). Root colonization and spore density were increased by both fungus species and fertilizer, which interacted (Table 7). Colonization percentages of all AM plants with fertilization were higher than without fertilization. The percentages were high, ranging from 38.4-79.5%, with the highest percentage found in *A. elegans* plants. Spores in fungal treatments with fertilization, generally recovered in higher number than without fertilization. The spore number ranged from 16.1-74.6 spores/100 g soil and the highest density was found in mixed AM species with fertilization.

DISCUSSION

Pot cultures, using host plants grown in soil diluted with sterile sand, are most commonly used to propagate AM fungi (Brundrett *et al*., 1996). In our present study, all 6 indigenous AM fungi were recovered from all 5 host plant pot cultures. Variation in spore density and mycorrhizal colonization

was increased by host plants and AM fungi. Results presented here for spore density and root colonization are in agreement with those found in the greenhouse and the field showing that AM species, host plant species and soil conditions have been reported to effect on mycorrhizal formation and sporulation in pot cultures (Brundrett *et al*., 1996; Liu and Wang, 2003). From our observation, most plants inoculated with small to medium sized spores of *Glomus* and *Acaulospora* species was generally more successful than inoculation with the larger sized spores of *Scutellospora* species. The spore abundance must be related to their sporogenous characteristics. It has been reported that *Glomus* and *Acaulospora* species usually produce more spores than *Gigaspora* and *Scutellospora* species in the same environment conditions, because smaller spores require a short time to produce spores than large spores (Hepper, 1984; Bever *et al*., 1996). The high success rates of spore density and colonization percentage of at least 3 of 6 AM species were observed on sorghum and maize suggested that these plants are favorable hosts especially for *A. elegans*, *G. etunicatum* and *G. mosseae* compared to other hosts tested. Thus, sorghum and maize pot culture-produced spores as inoculum in P-deficient soil medium are more suitable for large scale production as well as for research purposes and nursery practice.

In the greenhouse experiment, growth of non-AM plants was very stunted in P-deficient soil medium (available P 1.4 ppm). Applying additional P fertilizer to non-AM plants could enhance growth of plants. However, even with the different allotments of P fertilizer, all AM species greatly stimulated plant growth with bigger size than non-AM plants in this unsuitable soil condition. Enhancement effects on plant growth, tended to increase with increasing P rates and more efficiency varied with the different kinds of AM species. Phosphorus responses for the plant growth agreed with that reported for other AM plants grown in a controlled environment (Siqueira *et al*., 1998a; Youpensuk *et al*., 2005), thereby confirming mycorrhizal nutritional benefits and the strong interrelationship between P supply and mycorrhizal response under nutrient-stressed conditions. AM inoculation had slightly effects on seedling growth when plants received low P rates at planting, whereas strongly effects were found at higher P rates. The present results suggested that addition 250 mg P kg^{-1} to *C. acuminatissima* seedlings was suitable to produce either non-AM plants or AM plants in P-deficient soil. Although, non-AM plants at maximal P rate were higher than at minimal rate but still significantly lower than AM plants at the same P addition. The greatest height of AM plants was found in *G. etunicatum* plants with 250 mg P kg^{-1}, exhibited 1.8 fold over non-AM plants. Contrast with stem diameter of seedlings was not improved by the mycorrhizal symbiosis but diameter was also greatest with the highest P added, exhibited 1.5 fold increase over control with no P added. The Mycorrhizal Dependence (MD) of *C. acuminatissima* was high and trended to increase with applying fertilizer into P-deficient soil. In the absence of fertilizer, maximum MD exhibited 43.1% when fertilized with 150 mg P kg^{-1}, maximum MD exhibited 75.9% (data not shown). P-deficient plants lacking AM symbiosis tend to have a high root to shoot ratios usually associated with nutrient-stressed plants (Pacovsky *et al*., 1986). Whilst, root to shoot ratios of AM plants in our study were higher than for non-AM plants, especially in the absence of P fertilizer or low P rates, the higher ratios probably resulted in mycorrhizal stimulation of root growth for improving P acquisition under limiting P condition. Plants characterized as inefficient at acquiring soil P, may substantially improve P acquisition by morphological and physical adaptations include changes in P and dry matter partitioning that favor growth of roots over shoots and the induction of a high-affinity P uptake and transport system in roots during the development (Cogliatti and Clarkson, 1983; Marschner *et al*., 1996).

P contents in AM plants were significantly increased with levels of P application. AM fungi most likely increased nutrient uptake from the soil due to the external hyphae can exploring greater soil volume and delivering nutrients to the host plants (Joner and Jakobsen, 1995; Koide and Mosse, 2004). Root colonization of plant species in many greenhouse experiments is diminished by high soil

P availability and concomitant enhanced P concentration in plant tissues (Vaast *et al*., 1996; Youpensuk *et al*., 2005). Contrasting with this suppressive effect observed with AM colonization in *C. acuminatissima* was not differed by increasing P levels application while P status remained unaffected. This experiment showed that AM inoculation of *C. acuminatissima* seedlings produces large plants with improved P status, thus confirming the high AM-dependency of host plant. This study also indicates the tolerance abilities of selected AM species on P-application rates, resulting from their abilities to promote plant P accumulation.

In the nursery experiment, seedlings grown in forest soil medium with slow-release fertilizer applied were slightly bigger than controls. Most AM plants without fertilizer added grew poorly with growth parameters similar to those of non-AM plants. Plant height, shoot and root dry weights and shoot and root P contents were increased by fertilizer but not by mycorrhiza, whereas only stem diameter was increased by both factors. Higher stem diameter of *G. etunicatum* and mixed AM species plants with fertilization may result from direct fungal efficiency effects or fungal-fertilizer interactions in such soil condition. AM colonization and P concentrations of *C. acuminatissima* seedlings were quite high with slow-release fertilizer added. This may be fertilization effect on P accumulation through its influence on AM symbiosis. Heavy application of P fertilizer or sufficient P condition at planting may reduce mycorrhizal formation, sporulation and the MD of host and thus mycorrhizal effectiveness for the seedling growth (Siqueira *et al*., 1998b). From our observation, growth of AM plants grown in forest soil was less than grown in P-deficient soil. This may the result of plants responding well to mycorrhizas in low or moderate P soils are regarded as mycorrhizal dependent. That is, they depend on mycorrhiza to show their full potential (Haselwandter and Bowen, 1996). The components of forest soil medium (available P 15.8 ppm) were rich in both organic and inorganic nutrients. Thus, AM fungi may be loose their function on stimulating plant growth in this nutrient condition. High dissolved inorganic nutrients in tropical forest soil may make AM fungi unnecessary to meet nutrient (Maffia *et al*., 1993). The consistent effects of AM fungi on plant growth were diminished or disappeared with nutrient abundance in the soil. Strongly mycorrhizal effects on external P requirement for maximal growth of seedlings were high and consistent in nutrient poor soil but were diminished and varied unpredictably with levels of fertilizer and P requirement of individual plant species. Phosphorus is not only a suppressed factor on AM symbiosis. Further, Youpensuk *et al*. (2005) reports that application of high rates of P or N can depressed AM colonization and spore formation. *C. acuminatissima* seedling grown in forest soil medium with nutrient abundance did not respond to AM inoculation although root colonization was high. Without fertilizer added, all AM plants were still equal size with non-AM plants. It may be resulted in reduction of AM symbiosis caused decreasing nutrient uptake ability for growth of mycorrhizal plants.

In addition, mycorrhizal inoculation with selected AM fungi and application of optimal P rate on early plant development are highly advantageous for high quality sapling production in forest tree nurseries and sapling establishment in low nutrient soils in forest restoration areas in Thailand. All AM species tested were greatly effective in promoting growth parameters of AM seedling in nutrient poor medium, but diminished their function in nutrient abundant medium. Differential responses of AM seedlings in these experiments appear to be related to nutrient available (inorganic and organic forms) in medium and form of fertilizer (easy soluble or slow-releasing) by plant and AM fungi. Therefore, the success of AM technology will depend upon dependent mycorrhizal host, optimal soil condition and well-adapted effective fungal strains.

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