CHAPTER 7

EFFECTIVENESS OF AMF IN DIFFERENT TYPES OF INOCULUM ON COWPEA GROWING IN ACID SOIL

7.1 Introduction

The low root colonization and low AMF response in chapter 6 compared with chapter 5 might be caused by the different inoculum types. In chapter 5 mix-species soil inoculum was used while in chapter 6 plants were inoculated single species spore inoculum. The diversity of AMF species in soil inoculum is an advantage point of soil inoculum because host plant has a chance to choose the AMF that it prefers (Bethlenfalvay, 1992). Moreover soil inoculum consist of 3 kinds of infection unit including AMF spore, infected root fragment and external hyphae in soil while spore inoculum has only AMF spore as an infection unit (Lui and Luo, 1994). Inoculation method is an important factor. As described in chapter 6, the AMF spores in soil inoculum had better chance to infect root because spores inoculum was lied on filter paper that make spore could not distribute in soil profile. But the disadvantage point of mixed species soil inoculum are 1) the effective AMF species could not be identified 2) the result could be influenced by the other micro organisms in the soil. For example *Streptomyces sp.* AcH 505 was reported to stimulate hyphe growth and root colonization of ectomycorrhiza *Amanita muscaria* in spruce (Schrey *et al.*, 2007). *Pseudomonas monteilii* stain HR13 enhance root colonization of *Glomus intraradices* in *Acacia holosericea* and dual inoculation of *G. intraradices* and *P. monteilii* stimulated acacia

growth much more than inoculating with *G. intraradices* alone. To evaluate the effective ness of different inoculum types three pot experiment were conducted. The first experiment had objectives to 1) find effective inoculum type to enhance growth in acid soil 2) determine effects of the rate for inoculum in each type. The objective of the second experiment was explore how surface sterilization of AMF spores affect their ability to colonize the host's roots. The last experiment was to compare effectiveness of the AMF inoculum as pure culture of spores and a host plant's rhizosphere soil.

7.2 Materials and methods

Growth medium preparation

The growth medium was a mixture of soil and sand (2:1 w/w). The growth medium pH was adjusted to 5 by adding $Al_2(SO_4)_3$ $18H_2O$. Phosphorus concentration in growth medium was adjusted to 11 mg P/kg by adding 56 mg KH_2PO_4/ kg. Basal nutrients were as follows (mg/kg): K_2SO_4=71, $CaCl_2.H_2O$=94, $MnSO_4.H_2O$=10, $ZnSO_4.7H_2O$=5, $CuSO_4.5H_2O$=2.1, H_3BO_3=0.8, $CoSO_4.7H_2O$=0.36 and $Na_2MoO_4.2H_2O$=0.18. The prepared growth medium was autoclaved at 121°C for one hour.

Harvesting

At each harvest shoot was cut at soil surface then oven dried before weighed. Roots were washed to free from soil. Root nodules were corrected, counted and oven dried before weighed. Fresh roots were weighed before cutting into 1 cm fragments. Then a root sub-sample (10% of total root fresh weight) was randomly taken from each pot for AMF measurement. The remained roots were oven dry before weighed.

Root colonization measurement

For AMF measurement root samples were cleared in 10% KOH for several days before staining with 0.05% trypan blue in lactoglycerol. Thirty centimeter of root fragments was randomly taken from each sample for microscopy side. Root colonization percentage was assessed using the intercept method (Brundrett *et al.* 1996) under a compound microscope. The root colonization percentage was arsine transformed before analysis of variance.

Phosphorus analysis

Shoot and root P concentration were measured by Molybdovanadate-Phosphoric Acid method (Murphy and Riley, 1962). Soil P was measured by BrayII method.

7.2.1 Effects of different AMF inoculum types on cowpea in acid soil

A pot experiment was conducted in a glasshouse at Chiang Mai University in June 2009 (early raining season). The plant used was cowpea (*Vigna unguiculata* L. Walp.cv. Ubon Rajathanee). Seeds were surface sterilized by soaking in 70% ethanol for 5 minute then washed by sterilized water. Six surface sterilized seed were sown in 5 L drained plastic pot containing 3.6 kg of growth medium. There were 3 holes in the surface soil in a pot and 2 seeds were sown in each hole. Seeds in each hole were inoculated with different inoculum. The inoculum types included 1) 10 g of infected *Macaranga denticulata* root fragment (RF) 2) 10 g of soil from *M. denticulata* root zone (Ma) containing 100, 250 or 500 spores (Ma100, Ma250 and Ma500 respectively) 3) 10 g of soil from mimosa (*Mimosa invisa*) root zone (Mi) containing 100, 250 or 500 *Acaulospora morrowiae* CMU22 (the Am2 in chapter 6, Figure 6.1b) spores (Mi100, Mi250 and Mi500 respectively) 4) 100, 250 or 500 *Acaulospora morrowiae* CMU22 spores extracted from Mi (Ac100, Ac250 and Ac500 respectively). Un-inoculated cowpeas were used as control. Each seed was inoculated with 1 ml of appropriate rhizobium suspension at 10^9 cells/ml (provided by Department of Soil Science Faculty of Agriculture Chiang Mai University). Seedling emerged at 3 days after sowing. Plants were thinned to 3 plants per pot at 1 week after emergence. The experiment was designed in Randomize Complete Block Design (RCB) with 11 inoculation treatments and 3 replications. A pot was an experimental unit. Three set of pots with complete treatments and replications were set up for 3 harvests at 15, 35 and 46 days after emergence (V4, flowering and pod filling stage respectively).

The soil inoculum (Ma) and root fragments (RF) were collected from the root zone of 6 mounts old *M. denticulata* growing in 5 L plastic pot containing soil from Tee Cha village. One gram of Ma contained 68 AMF spores of mixed species, which was diluted in autoclaved Ma soil for Ma100, Ma250 and Ma500. The mimosa inoculums (*Mi*) were *Acaulospora morrowiae* CMU22 propagated for 5 months in the root zone of mimosa growing in 5 L plastic pot containing low P (11 mg P/kg) acidic soil (pH 5). The *Acaulospora morrowiae* CMU22 was isolated from a single spore in soil inoculum that used in chapter 5 by useing *Mimosa invisa* as a host plant. One gram of *Mi* contained 103 spores *Acaulospora morrowiae* CMU22, was diluted in autoclaved Mi for Mi100, Mi250 and Mi500. Plants were harvested at 15, 35 and 46 day after emergence.

7.2.2 Effectiveness of surface sterilized AMF spores on mimosa

A pot experiment, with 7 inoculating treatments and 4 replications in a randomized complete block design with 4 replications, was conduct in a greenhouse at Chiang Mai University from August to September 2009. The test plant was mimosa (*M. invisa* Mart). Seeds were surface sterilized by soaking in 70% ethanol for 5 minutes then washed by sterilized water before sowing. Twenty surface sterilized seeds were sown in 0.5 L drained plastic pot containing 500 g of growth medium. The seeds were inoculated with AMF spores (*Acaulospora morrowiae* CMU22, the AM2 in chapter 6, figure 6.1b) that had been surface sterilized by 0.5% NaClO for 1 minute or 0.2% chloramines-T for 5 minutes. Un-inoculating plants and plants inoculated with spores that had not been surface sterilized were used as controls. The rate of spores in inoculated treatments was

varied at 100 and 300 spores per plant. Seeds in each pot were inoculated with effective rhizobium strain. Plants emerged at 4 days after sowing, and were thinned to 5 plants per pot. One pot was one experimental unit. There was one set of pots with complete treatments and replications for each of the 3 harvests at 14, 25 and 35 days after emergence to measure root colonization, root fresh weight and above ground dry matter.

7.2.3 Effects of different AMF inoculum types on cowpea and mimosa in acid soil

Four treatments of inoculum types were applied to mimosa (*Mimosa invisa* Mart) and cowpea (*Vigna unguiculata* L. Walp.cv. Ubon Rajathanee) in 3 replications, arranged in randomized complete block design. A 5 L plastic pot containing 3.6 kg of growth medium was an experimental unit. The growth medium had pH 5 and contained 11 mg P/kg (Bray II). Pots were inoculated with 4 different inoculum types. The treatments of AMF inoculum types were 1) 22 g of soil from the root zone of *Macaranga denticulata* growing in farmer's field at Huai Teecha, containing 1,500 spores of mixed AMF species (Ma) 2) extracted spore from the same weight of the soil from *M. denticulate* plus 22 g autoclaved the soil inoculum (Ma-spores); 3) 1500 spore of *Acaulospora morrowiae* CMU22 (Ac-spores, the AM2 in chapter 6, figure 6.1b) plus 22 g autoclaved the soil inoculum; and 4) 22 g of autoclaved soil inoculum as a control treatment. All extracted spores were surface sterilized by soaking in NaClO (0.5%) for one minute before inoculation. Seeds of the 2 legumes were surface sterilized with 70% ethanol for 5 minutes and washed 3 times with sterilized water before sowing. Twenty seeds of mimosa or 5 seeds of cowpea were sown in each pot. Appropriate rhizobium inoculation

(10^9 cells/ml suspension) was applied at the rate of 1 ml/seed for cowpea and 0.5 ml/seed for mimosa. For inoculum preparing, the Ma was the same soil inoculum as Ma in experiment 7.2.1. The inoculum Ac-spore was the extracted spore from Mi soil inoculum in experiment 7.2.1.Cowpea seedlings were thinned to 3 per pot and mimosa seedlings to 10 per pot at 11 days after sowing. One complete set of treatments and replications was harvested at 39 day after sowing and set at 59 days. Data of each legume was analyzed separately. Data in percentage was arsine transform before analized. In the second harvest data of shoot root and total dry weight was transformed by log before analysis of variance.

7.3 Result

7.3.1 Effects of different AMF inoculum types on cowpea in acid soil

Some effects of AMF were evident with RF and Ma at 15 days (Table 7.1 and 7.2). At this early stage, 1.1% root colonization was found in control treatment, but significantly higher at 7% RF. In Ma increasing spore rate from Ma100 to Ma500 increased root colonization from 4.6 to 15.1%. Root colonization in Mi and Ac were not distinguishable from uninoculated control, even at the maximum rate of Mi500 and Ac500. At 15 days, there was no effect of AMF inoculation on cowpea nodulation and growth (Table 7.1 and 7.2).

Table 7.1 Root colonization shoot root and total dry weight of cowpea inoculated with different inoculun types varied spore rate at 15 days after emergence

Treatment	Root colonization (%)	Total biomass (g/plant)	Shoot biomass (g/plant)	Root biomass (g/plant)
Control	1.1 D	0.61	0.37	0.24
RF	7.1 ABC	0.54	0.32	0.22
Ma100	4.6 CD	0.50	0.26	0.24
Ma250	10.6 AB	0.53	0.29	0.24
Ma500	15.1 A	0.59	0.37	0.22
Mi100	3.0 BCD	0.59	0.35	0.24
Mi250	1.5 D	0.64	0.37	0.27
Mi500	1.4 D	0.56	0.34	0.23
Ac100	0.2 D	0.55	0.31	0.24
Ac250	0.6 D	0.49	0.29	0.20
Ac500	1.6 D	0.63	0.36	0.27
F-test	**	NS	NS	NS

** = significant different ($P < 0.01$), NS = non-significant different, mean in the same column followed by different letter indicate significant different ($P < 0.05$)

Shoot P concentration in RF, Mi and Ac treatments were little different from control and no effect of spore rates was detected. However, shoot P concentration was increased significantly by increasing spore rate in Ma. To increase shoot P 250 spore of Ma was needed. In Ma250 shoot P concentration was 43% higher than control. But, increasing spore rate from Ma250 to Ma500 could not increased shoot P (Table 7.2). Total P content in all inoculated treatment was not higher than control. The P content in RF, Mi and Ac treatments was not different and no effect of spore rate was found (Table 7.2). In Ma treatments increasing spore rate continually increased P content and the P content in Ma500 was higher than the other inoculated treatment (Table 7.2).

The P uptake per unit root weight was used to indicate P uptake efficiency. Phosphorus uptake efficiency in RF, Mi and Ac treatment were not higher than in control and no effect of spore rate was found (Table 7.2). In Ma, increasing spore rate from Ma100 to Ma500 continually increased P uptake but the P uptake of Ma100 and Ma250 was not higher than control. Only in Ma500 that P uptake was 18% higher than control (Table 7.2).

Table 7.2 Nodule number, shoot P concentration and P uptake per unit root weight of cowpea inoculated with different inoculum types varied spore rate at 15 days after emergence

Treatment	Nodule number per plant	Shoot P concentration (% w/w)	Total P content (mg/plant)	P uptake per unit root weight (mg/g)
Control	24.0	0.14 CDE	0.96 ABC	4.17 BC
RF	47.8	0.13 DE	0.73 D	3.39 DE
Ma100	37.0	0.16 BC	0.81 CD	3.55 CDE
Ma250	30.6	0.20 A	1.03 AB	4.20 B
Ma500	32.0	0.18 AB	1.08 A	4.92 A
Mi100	24.0	0.11 E	0.81 CD	3.47 DE
Mi250	30.0	0.13 DE	0.88 BCD	3.32 E
Mi500	31.0	0.12 DE	0.79 CD	3.53CDE
Ac100	29.9	0.15 CD	0.84 CD	3.65 BCDE
Ac250	19.1	0.14 CDE	0.79 CD	3.99 BCD
Ac500	21.1	0.12 DE	0.89 BCD	3.46 DE
F-test	NS	*	*	*

* = significant different ($P < 0.05$), NS = non-significant different, mean in the same column followed by different letter indicate significant different ($P < 0.05$)

At 35 days after emergence, there was still no root colonization in control treatment, but the AMF treatments became more clearly distinguishable by both type and rate of inoculation. In general, the cowpea roots have become colonized to a much greater extent than at 15 days in all AMF inoculated treatments. The highest root colonization was found in RF (86.1%) that was higher than the other treatment except Ma500 (73.8%). Root colonization in Mi and Ac treatment were same in every spore rate. Increasing spore rate had no effect on root colonization in all inoculum types (Table 7.3). AMF inoculation increased plant dry weight, nodulation and P uptake, with the greater effects at higher rate of inoculation (Table7.3). Inoculating with RF increased total dry weight for 102% (Table 7.3). The Ma distinctly increased total dry weight, with the greatest effect at Ma500. Total dry weight in Ma100, Ma250 and Ma500 was 96, 153 and 194 % higher than the control (Table 7.3). Total dry weight was increased by Mi too but the Mi was less effective comparing with the Ma. Total dry weight was 67, 76 and 114 % increased by inoculating with Mi100, Mi250 and Mi500 respectively but no effect of spore rate was found because total dry weight between different spore rates was not significant different. Total dry weight in Ac was same as Mi in every spore rate but Ac100 and Ac250 could not increase total dry weight. Only dry weight in Ac500 that was 100% higher than control. The same response were found in shoot and root dry weight (Table 7.3).

Table 7.3 Root colonization, shoot root, and total dry weight of cowpea inoculated with different inoculum types with varied spore rate at 35 days after emergence

Treatment	Root colonization (%)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Total dry weight (g/plant)
Control	0 E	0.74 E	0.43 E	1.17 E
RF	86.1A	1.55 BCD	0.81 ABC	2.36 BCD
Ma100	61.8 BCD	1.54 BCD	0.76 ABCD	2.30 BCD
Ma250	67.8 BC	2.11 AB	0.85 AB	2.96 AB
Ma500	73.8 AB	2.48 A	0.95 A	3.43 A
Mi100	55.0 BCD	1.36 CD	0.59 CDE	1.94 CD
Mi250	56.7 BCD	1.42 CD	0.63 BCDE	2.05 CD
Mi500	59.8 BCD	1.80 BC	0.71 ABCD	2.50 BC
Ac100	46.2 D	1.08 DE	0.53 DE	1.62 DE
Ac250	52.0 CD	1.28 CDE	0.54 DE	1.82 CDE
Ac500	56.6 BCD	1.74 BC	0.71 ABCD	2.44 BC
F-test		**	*	**

*, ** = significant different $P < 0.05$ and $P < 0.01$ respectively, NS = non-significant different, mean in the same column followed by different letter indicate significant different ($P < 0.05$)

At this stage, AMF inoculation had no effect on nodule number (Table 7.4). For nodule dry weight, all kind of inoculum types except RF increased nodule dry matter and no effect of spore rate was found (Table 7.4). Shoot P concentration was increased by RF, Mi and *Ac* inoculation but not by Ma, with little difference between spore rates. All kinds of inoculation treatment increased total P content. The total P content in RF, Mi and Ac treatments was same and no effect of spore rate was found but in Ma treatments increasing spore rate increased the P content (Table 7.4). All types of AMF inoculum increased P uptake per unit root weight, but with little difference between spore rates in each type (Table 7.4).

Table 7.4 Nodule number, nodule dry weight, shoot P concentration and P uptake per unit root weight of cowpea inoculated with different inoculum types with varied spore rate at 35 days after emergence

Treatment	Nodule number per plant	Nodule dry weight (mg/plant)	Shoot P concentration (% w/w)	Total P content (mg/plant)	P uptake/unit root weight (mg/g)
Control	51	8 C	0.096 E	1.11 C	2.6 C
RF	80	36 BC	0.130 BCD	3.30 AB	4.2 AB
Ma100	80	50 AB	0.116 DE	2.93 B	3.9 BC
Ma250	88	64 AB	0.122 BCDE	3.84 AB	4.5 AB
Ma500	76	76 A	0.116 CDE	4.28 A	4.6 AB
Mi100	87	52 AB	0.160 A	3.15 AB	5.4 AB
Mi250	96	52 AB	0.145 ABC	3.08 AB	4.9 AB
Mi500	83	51 AB	0.149 B	3.77 AB	5.7 A
Ac100	73	40 B	0.160 A	2.64 B	5.0 AB
Ac250	80	50 AB	0.150 AB	2,84 B	5.3 AB
Ac500	82	53 AB	0.124 BCDE	3.31 AB	4.7 AB
F-test	NS	*	**	**	*

** = significant different $P < 0.01$, * = significant different $P < 0.05$, NS = non-significant different, mean in the same column followed by different letter indicate significant different ($P < 0.05$)

At 46 days after emergence, root colonization of 0.2% was found in control treatment, and much higher in the AMF inoculation treatment (Table 7.5). The higher root colonization percentage was found in RF and Ma treatments and lower in Mi and Ac treatments, and there was no effect of spore rate on root colonization with all types of inoculum (Table 7.5). All types of AMF inoculation increased total dry weight, but with the strongest effect from Ma500. In Ma treatment increasing spore rate increased total dry weight. Plants inoculated with RF, Mi and Ac had same total dry weight and no effect of spore rate was found in these treatments (Table 7.5). The same response was found in shoot dry weight (Table 7.5). All types of inoculum enhanced root biomass production. Inoculating with RF increased root dry weight for 149%. Root dry weight was increased by Ma too and the effect was bigger in higher spore rate. Root dry weight in Ma100, Ma250 and Ma500 was 89, 128 and 181% higher than control respectively. Inoculating with Mi increased root dry weight but there was no effect of spore rate because root weight in all 3 spore rates was not different. Root dry weight in Ac and Mi in each spore rate was not different (Table 7.5).

Table 7.5 Root colonization, shoot root and total dry weight of cowpea inoculated with different inoculum types with varied spore rate at 46 days after emergence

Treatment	Root colonization(%)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Total dry weight (g/plant)
Control	0.2 E	0.91 C	0.56 E	1.47 C
RF	82.6 A	2.07 B	1.40 AB	3.48 AB
Ma100	70.0 ABC	1.94 B	1.06 BCD	3.00 B
Ma250	73.5 AB	2.76 AB	1.28 ABC	4.05 AB
Ma500	78.5 A	3.16 A	1.58 A	4.74 A
Mi100	51.4 CD	1.99 B	0.98 CD	2.97 B
Mi250	53.2 BCD	2.32 AB	0.99 CD	3.31 B
Mi500	49.4 D	2.14 AB	1.11 BCD	3.25 B
Ac100	56.7 BCD	2.21 AB	0.87 DE	3.08 B
Ac250	46.3 D	2.36 AB	1.04 BCD	3.40 B
Ac500	53.5 BCD	2.03 B	1.18 BCD	3.21 B
F-test	**	*	**	*

** = significant different $P < 0.01$, * = significant different $P < 0.05$, NS = non-significant different, mean in the same column followed by different letter indicate significant different ($P < 0.05$)

All types of inoculum increased nodule number, but nodule number was increased with rate of inoculum in Ac (Table 7.6). The RF treatment increased nodule number for 146% comparing with control, which was the same as Ac100, but significantly more nodules in Ac500 (Table 7.6). Even more nodules were produced in RF and Ma, but increasing the rate in Ma did not increase nodule number (Table 7.6). All types of AMF inoculation increased nodule dry weight ten folds or more, with clear enhancing effect of rate in Ma and Ac (Table 7.6). Nodule dry weight in RF treatment was 10 times of control. The nodule dry weight in RF was not different with Mi treatment and no effect of spore rate was found (Table 7.6). In Ma, increasing spore rate from Ma100 to Ma500 continually increased nodule dry weight. The nodule weight in Ma100, Ma250 and Ma500 were 9, 13 and 16 times of control (Table 7.6). Every type of AMF inoculation increased shoot P concentration and no effect of spore rate was found (Table 7.6). All kinds of inoculum increased total P content. The P content in RF, Mi and Ac treatments was same and not effected by spore rate. But in Ma treatments increasing spore rate increased P content and the P content in Ma500 was higher than the other treatments. The P uptake per unit root weight was enhanced by AMF inoculation but no effect of spore rate was found.

Table 7.6 Nodule number, nodule dry weight, shoot P concentration and P uptake per unit root weight of cowpea inoculated with different inoculum type with varied spore rate at 46 days after emergence

Treatment	Nodule number per plant	Nodule dry weight (mg/plant)	Shoot P concentration (%)	Total P content (mg/plant)	P uptake per unit root (mg/g)
Control	36 F	7.7 D	0.085 C	1.26 D	2.6 C
RF	90 E	80.9 C	0.128 AB	4.17 BC	4.2 AB
Ma100	117 ABCD	72.8 C	0.127 AB	3.76 C	3.9 BC
Ma250	102 BCDE	95.9 ABC	0.124 AB	4.97 AB	4.5 AB
Ma500	101 CDE	121.2 A	0.120 BC	5.68 A	4.6AB
Mi100	127 AB	86.3 BC	0.143 AB	4.09 BC	5.4 AB
Mi250	120 ABC	90.1 BC	0.157 A	4.88 AB	4.9 AB
Mi500	113 ABCDE	80.4 C	0.131 AB	4.11 BC	5.7 A
Ac100	98 CDE	82.0 C	0.162 A	3.91 BC	5.0 AB
Ac250	93 DE	85.3 BC	0.129 AB	4.26 BC	5.3 AB
Ac500	129 A	110.7 AB	0.140 AB	4.20 BC	4.7 AB
F-test	**	**	*	**	*

** = significant different $P < 0.01$, * = significant different $P < 0.05$, mean in the same column followed by different letter indicate significant different ($P < 0.05$)

7.3.2 Effectiveness of surface sterilized AMF spores on mimosa

At 14 days, no root colonization was found in all treatment and there was no effect of AMF on mimosa growth (Table 7.7). At 25 days, root colonization was not found in control treatment. In inoculated treatments root colonization was low ranging from 1.9 to 6.9% and no effect of surface sterilization of the spores or spore rate on root colonization. Growth was not affected by AMF inoculation (Table 7.7). At 35 days, mimosa in control treatment still had no root colonization. Mimosa inoculated with surface or un-surface sterile spore had same root colonization and no effect of spore rate was found. The root colonization ranged between 19 and 26%. The AMF inoculation still had no effect on mimosa growth (Table 7.7).

Table 7.7 Root colonization, shoot dry weight and root fresh weight of mimosa inoculated with AMF spores sterilized with different antiseptic at 14, 25 and 35 days

Antiseptic treatment	Inoculation rate (spore/plant)	Shoot dry weight (mg/plant)	Root fresh weight (g/plant)	root colonization (%)
			14 days	
uninoculated		12	87.6	0
nonsterilized	100	12	86.1	0
	300	13	94.1	0
NaClO	100	12	92.3	0
	300	11	72.7	0
Chloramine	100	12	87.0	0
	300	10	67.8	0
F-test		NS	NS	NS
			25 days	
uninoculated		13	137.6	0 b
nonsterilized	100	17	155.7	4.3 a
	300	18	157.6	5.9 a
NaClO	100	17	159.1	6.9 a
	300	18	155.7	6.7 a
Chloramine	100	17	157.3	3.8 a
	300	16	141.6	1.9 ab
F-test		NS	NS	*
			35 days	
uninoculated		21	213.3	0 b
nonsterilized	100	30	268.7	19.9 a
	300	29	248.1	21.1 a
NaClO	100	33	244.0	26.2 a
	300	38	275.6	20.8 a
Chloramine	100	26	203.2	20.7 a
	300	33	256.9	24.1 a
F-test		NS	NS	**

NS = non-significant different, * = significant at $P < 0.05$, ** = significant at $P < 0.01$, mean in the same column followed by different letter indicate significant different ($P < 0.05$)

7.3.3 Effectiveness of different AMF inoculum types on cowpea and mimosa in acid soil

At 39 day after sowing, root colonization was not found in un-inoculated control treatment of both cowpea and mimosa. Inoculating with Ac-spore and Ma gave the same root colonization (16 and 29% in cowpea and 12 and 21% in mimosa respectively; Table 7.8). Inoculating with Ma-spore gave very low root colonization and was not significantly different from the control treatment (0.8% in cowpea and 1.4% in mimosa) (Table 7.8). At this stage all types of AMF inoculation had no effect on biomass yield and nodulation of cowpea (Table 7.8 and 7.9). In mimosa, shoot, root and total dry weight of Ma inoculated plant were 4, 2 and 3 times of control respectively but the effect on nodulation was not found (Table 7.8 and 7.9). The other types of inoculum had no effect on mimosa growth and nodulation (Table 7.8 and 7.9).

Table 7.8 Root colonization, shoot root and total dry weight and nodule number per plant of cowpea and mimosa inoculated with different inoculum types at 39 days after sowing

	Root colonization (%)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	Total dry weight (g/plant)	Nudule number per plant
			Cowpea		
Control	0.0 B	0.86	0.51	1.37	67.9
Ac-spore	15.6 A	0.71	0.41	1.13	70.4
Ma	29.3 A	0.96	0.53	1.49	80.8
Ma-spore	0.8 B	0.63	0.40	1.03	68.1
F-test	**	NS	NS	NS	NS
			Mimosa		
Control	0.0 C	0.03 B	0.03 B	0.06 B	3.0
Ac-spore	21.0 A	0.04 B	0.02 B	0.07 B	5.6
Ma	11.8 AB	0.13 A	0.07 A	0.20 A	14.3
Ma-spore	1.4 BC	0.03 B	0.03 B	0.06 B	4.0
F-test	**	**	**	**	NS

** = significant different ($P < 0.01$), NS = non-significant different, mean in the same column followed by different letter indicate significant different ($P < 0.05$)

At this stage shoot P concentration, total P content and P uptake per unit root weight of cowpea were not affected by AMF inoculation (Table 7.9). Although shoot P concentration of mimosa was not affected by AMF, inoculating with Ma increased total P content for 85%. Inoculating with Ac-spore and Ma increased P uptake per unit root weight of mimosa for 86 and 55% respectively while inoculating with Ma-spore had no effect on total P content and P uptake per unit root weight (Table 7.9).

Table 7.9 Nodule dry weight, shoot P concentration, total P content and P uptake per unit root weight of cowpea and mimosa inoculated with different inoculum types at 39 days after emergence

	Nodule dry weight (mg/plant)	Shoot P concentration (%)	Total P content (mg/plant)	P uptake per unit root weight (mg/g)
		Cowpea		
Control	7.13	0.080	1.16	2.30
Ac-spore	8.00	0.102	1.25	3.08
Ma	13.43	0.094	1.53	2.93
Ma-spore	6.23	0.094	1.01	2.53
F-test	NS	NS	NS	NS
		Mimosa		
Control	0.20	0.125	0.07 B	2.71 B
Ac-spore	0.37	0.176	0.13 B	5.06 A
Ma	2.17	0.153	0.31 A	4.21 A
Ma-spore	0.00	0.139	0.07 B	2.72 B
F-test	NS	NS	**	**

NS = non-significant different, ** = significant different ($P < 0.01$), mean in the same column followed by different letter indicate significant different ($P < 0.05$)

At 59 days, in un-inoculated control treatment cowpea had a little root colonization (0.4%). Root colonization of cowpea inoculated with Ac-spore, Ma and Ma-spore was not different (36.5, 53.6 and 47.3% respectively; Table7.10). Mimisa had no root colonization in control treatment. Inoculating with Ac-spore, Ma and Ma-spore increased root colonization in mimosa to about the same extent at 32.8-46.2% (Table 7.10). Inoculating with Ma-soil or Ac-spore significantly increased shoot and total dry weight of both cowpea and mimosa but not when inoculated with Ma-spore (Table 7.10). Cowpea and mimosa root dry weight was increased only by Ma while inoculating with the other types of inoculum had no (Table 7.10). No effect of AMF on nodulation was found in cowpea. Mimosa nodule number was increased by Ma but not by the other inoculums (Table 7.10). Mimosa nodule dry weight was increased equally by Ma and Ac-spore but not by Ma-spore (Table 7.11).

Table 7.10 Root colonization, biomass yield, nodulation, shoot P concentration and total P content of cowpea and mimosa inoculated with different AMF inoculums at 59 days after sowing

	Root colonization (%)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	total dry weight (g/plant)	Nudule number per plant
			Cowpea		
Control	0.4 B	1.25 B	0.72 BC	1.97 B	145
Ac-spore	36.5 A	2.09 A	1.00 AB	3.09 A	150
Ma	53.6 A	1.85 A	1.09 A	2.94 A	144
Ma-spore	47.3 A	1.08 B	0.56 C	1.65 B	176
F-test	**	*	*	*	NS
			Mimosa		
Control	0.0 B	0.09 B	0.06 B	0.15 B	3 B
Ac-spore	32.8 A	0.31 A	0.16 AB	0.47 A	43 AB
Ma	46.2 A	0.43 A	0.28 A	0.71 A	85 A
Ma-spore	42.7 A	0.13 B	0.06 B	0.19 B	11 B
F-test	**	**	**	**	**

** = significant different ($P < 0.01$), mean in the same column followed by different letter indicate significant different ($P < 0.05$)

Shoot P concentration and P uptake per unit root weight of cowpea were not affected by AMF inoculation (Table 7.11). Total P contents, however, was about doubled in cowpea by Ac-spore and Ma but was not affected by Ma-spore (Table 7.11). Mimosa shoot P concentration was increased by 50% Ac-spore or Ma-spore. Total P content in mimosa was increased by 4-folds or more by Ac-spore and Ma-soil. The P uptake per unit root weight of mimosa was about doubled by inoculation with Ac-spore and 122% by Ma-spore.

Table 7.11 Nodule dry weight, shoot P concentration, total P content and P uptake perunit root weight of cowpea inoculated with different inoculum types at 59 days after emergence

	Nodule dry weight (mg/plant)	Shoot P concentration (%)	Total P content (mg/plant)	P uptake per unit root weight (mg/g)
		Cowpea		
Control	27.20	0.092	1.82 B	2.57
Ac-spore	55.40	0.120	3.91 A	3.89
Ma	80.80	0.109	3.41 A	3.15
Ma spore	41.50	0.095	1.75 B	3.21
F-test	NS	NS	**	NS
		Mimosa		
Control	0.03 B	0.12 B	0.17 B	2.78 B
Ac-spore	18.70 A	0.19 A	0.86 A	5.36 A
Ma	14.80 A	0.12 B	0.94 A	3.45 B
Ma-spore	1.70 B	0.19 A	0.39 B	6.23 A
F-test	*	**	*	**

** = significant different ($P < 0.01$), mean in the same column followed by different letter indicate significant different ($P < 0.05$)

7.4 Discussion

From the experiment 7.2.1, AMF from all types of inoculum colonized cowpea root successfully although in spore inoculum treatment (Ac). The root colonization of cowpea colonized by AMF from spore inoculum in chapter 6 was half of this experiment (Table 6.1, 6.3 and 6.5). Removing the filter paper under spore inoculum (Figure 6.2) doubled root colonization. This indicated that the filter paper in chapter 6 was a barricade agent distributing of spore in soil profile and depressed colonizing performance. AMF in Ma inoculum colonized plant root faster than the other inoculum types (Table 7.1). The RF and Ma inoculum had higher potential to colonized plant root than Mi and Ac that had the same potential (Table 6.1, 6.3 and 6.5). The potential to colonized host root did not relate with the effectiveness to promote plant growth. Although the most effective inoculum type in was Ma but the RF, Mi and Ac had same ability to promote cowpea growth. The hypothesis that "soil inoculum should be more effective that spore inoculum because it has 3 kind of inoculating unit (spore, infected root fragment and hyphe)" was rejected because the Mi and Ac had same potential to colonize root and promote plant growth. The result indicated that inoculum containing many fungi species had more potential to colonized root and promoting plant growth than single species inoculum. Bethlenfalvay (1992) suggest that inoculating with mixed inocula containing with different fungi should be more beneficial to host than single species inocula because plant has chance to choose their preference fungi or the more adaptable fungi in the mixed inoculum have chance to colonized plant root. The effect of spore rate on plant growth was found only in Ma. This indicated that only some fungi species in Ma are effective. The spore rate ranged from 100 to 500 spores per plant. The lowest spore rate (100

spore/plant) should be optimum for colonized and promote plant growth in single Mi and Ac (single species inoculum). But in Ma that containing many fungi species, the proportion of effective species in the inoculum might make the number of effective species spore lower than the rate 100 spore/plant. Therefore in Ma, increasing spore rate increased root colonization and plant growth (Table 7.3 and 7.5). But the Ma was produce from trapping spores in Teecha village soil. That was not a sterile condition and there were unidentified microorganism in the soil. The high root colonization and high growth of host plant might be the effect of the micro organism such as mycorrhiza helper bacterium in the soil (Bonfante and Anca, 2009). In the next experiment spores in Ma were extracted and surface sterilized to be used as spore inoculum for eliminating the effect of the other micro organism. But before that the suitable antiseptic for surface sterilizing has to be found out in experiment 7.2.2.

The result of experiment 7.2.2 showed that both NaOCl and chloramines T had no effect on colonizing performance of AMF spore (Table 7.7). But the NaOCl is in liquid form and easy to prepare. Therefore NaOCl was chosen for surface sterilizing AMF spore in experiment 7.2.3.

The Ac that was less effective than Ma in experiment 7.2.1 (Table 7.3 and 7.5) but in experiment 7.2.3, Ac-spore promoted plant growth as much as Ma (Table 7.10). This show the effectiveness of AMF inoculum did not depend on diversity of fungi species in the inoculum. The single species inoculum (Ac-spore) has the same effectiveness as the mixed species soil inoculum (Ma). In experiment 7.2.3 Ma was effective only in form of soil inoculum. When the spore of Ma was extracted to be used as the spore inoculum (Ma-spore) it could not promote plant growth and nodulation (Table 7.10 and 7.11). The

result indicated that there might be some microorganism such as mycorrhiza helper bacterium in Ma because only AMF propague in Ma (Ma-spore) could not promote plant growth (Bonfante and Anca, 2009). Effect of AMF on growth of cowpea and mimosa was different. In cowpea Ac-spore and Ma had the same effect. Both Ac-spore and Ma promoted cowpea growth only at 59 days (Table 7.10). In mimosa effect of Ma was shown earlier than Ac-spore. Ma had enhanced plant growth since 39 (Table 7.8) days while the effect of Ac-spore was seen at 59 days (Table 7.10). These results showed the effect of host species on mycorrhiza symbiosis. The higher growth was found in plant up taking more P. At 39 days Ma inoculation improved P uptake in mimosa. This caused mimosa in Ma treatment having higher growth than the others. At 59, Ma and Ac increased P uptake equally. This is the reason why Ma and Ac inoculation increasing plant growth equally.

The *Acaulospora morrowiae* CMU22 isolate was an effective AMF species to promote legume growth in acid soil. The effectiveness of inoculum did not depend on diversity of fungi species in the inoculum. In finally inoculating with single species spore and soil inoculum had same effective to promote legume growth. The effect of host plant species was shown that the response to AMF of mimosa showed earlier than the response of cowpea.

CHAPTER 8

GENERAL DISCUSSION

The indigenous AMF in shifting cultivation system of Haui Teecha village were associated not only with follow enriching tree like *Macaranga denticulata* (Yimyam *et al.,* 2003) but also with local legumes. In low P acid soil growth of legumes was benefiting from the association with AMF. Moreover modern cowpea lines introduced to this area were also benefiting from the association with indigenous AMF. Interestingly these modern lines are normally susceptible to acid soil but they could grow well with out P deficiency in low P acid soil in Haui Teecha. The hypothesis that "the indigenous AMF in Haui Teecha can help to alleviate acid soil stress in legumes" was accepted by results of pot experiments with steam sterilized soil.

In these experiments P deficiency was clearly the limiting factor of legume growth. Soil acidity depressed legume growth by accentuating P deficiency. The AMF alleviate acid soil stress by improving P uptake in legume. Therefore the benefit of AMF highly depended on soil P. Too high P application rate in experiment 4.2.2 and 6.2.1 eliminated benefit of AMF in cowpea. Host plant normally gets benefit from AMF only in stress condition and the benefit is diminished when the stress is alleviated (Marschner, 1995: Peng *et al.*, 1993). But too low soil P like in experiment 4.2.1 caused the negative effect of AMF on cowpea growth. The negative effect of AMF in extremely low soil P should be the result of P competition between host plant and AMF (Janos, 2007; Marschner, 1995).

The effectiveness of AMF highly depended on inoculating method. Pasting spores on filter paper that laying under seed is a practical method of AMF inoculation

in some report (Youpensuk *et al.,* 2006). This method is sometimes not suitable and it caused low root colonization and small response to AMF in experiment 6.2.3. Removing the filter paper in chapter 7 lifted root colonization and brought back high responsive to AMF of cowpea. This indicated that filter paper asked like a barrier blocking spores to distribute in to soil profile. This make AMF spores had less chance to infect plant root (Figure 6.2).

An *Acaulospora morrowiae* isolated from Haui Teecha soil is an effective species of AMF for cowpea. Pure culture of this species can stimulate legume growth as well as mixed species of the soil inoculum. The hypothesis that "soil inoculum should be more effective than spore inoculum because it contains many kinds of infection (spore, infected root fragment and hyphe)" was rejected. Soil and *Acaulospora sp* spore inoculum finally had same potential to colonized plant root and promote plant growth. But the AMF in soil inoculum from Haui Teecha colonized host roots faster and its effect showed earlier. This should be caused by another microorganism because when mix species spores were extracted from the soil inoculum and surface sterilized they fail to promote plant growth. It is not clear why the surface sterilized mixed specie spores extracted from Teecha soil failed to promote plant growth. It was not the result of the antiseptic killing the spore. The antiseptic (0.5% NaClO) was tested before in experiment 7.2.2. It has no effect on spore efficacy. And in the experiment 7.2.3 the extracted spores were still as effective in colonizing plant roots as soil inoculum even though it lost ability to promote plant growth. While spores of *Acaulospora morrowiae* that were surface sterilized as the same way were still effective to colonize and promote plant growth.

The one hypothesis that may be put forward is that soil inoculum from Macaranga root zone not only contain AMF but also contain another microorganism that promote effectiveness of AMF to promote host plant growth". Some kinds of soil bacteria can stimulate AMF root colonization and encourage benefit of AMF on plant growth. They are called mycorrhizal helper bacteria (MHR) (Bonfante and Anca, 2009). Some of them stimulate AMF root colonization by suppression of plant defense (Lehr et al., 2007). Frey-Klett et al. (2007) proposed a theory that MHB enhance mycorrhizal symbiosis by producing growth factors. These growth factors stimulate germination of AMF spore and mycelium growth.

This study proposed a new way to deal with soil acidity problem in legume by using AMF. There are some reports that indigenous AMF from the shifting cultivation system promote growth of some crop species including upland rice, sorghum, job's tears (Wongmo, 2008), rubber (Kanyasone, 2009) and coffee (Yimyam, 2006). But all of reports use soil inoculum collected from *Macaranga* root zone that contain mix species of AMF spore. The population of AMF associating with *M. denticulata* at Huai Teecha is diverse by year and season (Youpensuk et al., 2004). The effective AMF species was not identified. The quality of the soil inoculum might vary by time that it is corrected. There is an important question that "can we used another plant host for producing AMF inoculum instead of Macaranga". Macaranga is a local tree in the uplands; it difficult to grow in lowland environment and it take much time to growth (Yimyam, 2006b). In this study *Mimosa invisa* was the perfect host for multiplying AMF spore both for mixed species in non-sterilized soil in chapter 5 and single species isolate in sterile condition in chapter 6 and 7. Mimosa root was quickly and heavily colonized by AMF in chapter 7. The spores multiplied by mimosa were

effective to promote legume growth. The production of AMF inoculum is much easier by using mimosa as host plant. Therefore the use of mimosa as a host for producing AMF inoculum should be further investigated.

Local legumes growing in low P acid soil of shifting cultivation system of northern Thailand were highly associated with endogenous AMF. The AMF from the system was effective to alleviated acid soil stress in improve cowpea line when P was the limiting factor. The *Acaulospora morrowiae* isolated from Haui Teecha village was an effective species on cowpea.