

## 二種類の土壌水分条件下における根粒欠如アカシア類の成長および養分吸収に対するVA菌根菌接種効果

誌名	日本林學會誌 = Journal of the Japanese Forestry Society
ISSN	0021485X
著者	溝口, 岳男
巻/号	74巻5号
掲載ページ	p. 409-419
発行年月	1992年9月

## 論 文

## Effects of Inoculation of Vesicular-Arbuscular Mycorrhizal Fungi on Growth and Nutrient Uptake of Non-Nodulated *Acacia* spp. Seedlings in Two Soil Water Regimes

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MIZOGUCHI, Takeo : Effects of inoculation of vesicular-arbuscular mycorrhizal fungi on growth and nutrient uptake of non-nodulated *Acacia* spp. seedlings in two soil water regimes J. Jpn. For. Soc. 74 : 409~419, 1992 Non-nodulated seedlings of *Acacia melanoxylon* R. BR., *Acacia mangium* WILLD., *Acacia auriculiformis* A. CUNN. ex. BENTH were inoculated with or without vesicular-arbuscular mycorrhizal (VAM) fungi (*Glomus* spp.), and grown in a moist or a dry condition for three months. In place of a biosynthetic nitrogen supply, seedlings were equally fertilized. VA mycorrhizae were successfully established on every inoculated seedling. Height growth and biomass production of *A. melanoxylon* were not significantly affected by inoculation, though seedling morphology was affected. *A. mangium* responded positively at first; however, a foliar disease then occurred only on inoculated seedlings, after which the stimulative effects were nullified. *A. auriculiformis* showed some positive responses in dry matter production, but not in height growth. Totally, inoculation showed only slight growth stimulation both in the moist and the dry condition. In all cases, phosphorus concentrations of inoculated seedlings were significantly higher than control seedlings. For those legume trees, insufficient nitrogen supplies from nodules or external sources inhibit growth enhancement of VA mycorrhiza both in the moist and the dry condition, whereas phosphorus nutrition is greatly improved by VA mycorrhiza.

溝口岳男：二種類の土壌水分条件下における根粒欠如アカシア類の成長および養分吸収に対するVA菌根菌接種効果 日林誌 74 : 409~419, 1992 根粒を欠いた3種のアカシア (*Acacia melanoxylon*, *A. mangium*, *A. auriculiformis*) に対するVA菌根菌接種効果を調査するため、滅菌したマサ土にアカシアを播種し、一部にVA菌根菌を接種した。また、根粒の欠如に伴う窒素欠乏を補うため、窒素を主体とした施肥を行った。さらに、半数の苗の灌水を制限して乾燥ストレスを与えた。3カ月後、VA菌根はすべての接種苗に形成されていた。*A. melanoxylon* では接種により苗の形態が影響を受けたが、苗高・バイオマス生産への有意な影響は見られなかった。*A. mangium* では接種苗にのみ病害が発生し、初期の接種促進効果を減少させた。*A. auriculiformis* では接種によってバイオマス生産がやや向上したが、伸長成長への影響は小さかった。全体に、成長に対する接種効果は適潤・乾燥いずれの条件下でも小さかった。しかし、接種苗のリン濃度は非接種苗に比べ顕著に高くなっていた。これらの結果から、アカシア類に根粒が欠如している場合、その作用を部分的に窒素施肥で置き換えてもVA菌根菌接種の効果は十分には得られないことが明らかになった。

### I. Introduction

Leguminous trees have been widely introduced in various forests and revegetation areas, because they ordinarily grow fast and well adapt to infertile and harsh conditions. Those abilities are partly maintained by symbiotic relationships between plants and microorganisms. In a natural condition, two types of symbiotic organs, nodules and mycorrhiza, are commonly recognized on legume roots. It is well known that most of the leguminous plants deeply depend on nodules for their nitrogen demand. Therefore, nodules and

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their function (nitrogen fixation) are mostly principal components for maximum growth of legumes. On the other hand, it is also well documented that mycorrhiza plays an important role for plant growth and nutrition (1, 26). While the magnitude of mycorrhizal effects is various between plant species, leguminous plants often show striking growth improvement by a mycorrhizal inoculation (7). This growth acceleration is mainly derived from increment of nodule formation and nodule activities (35, 37). Generally, mycorrhizae ameliorate phosphorus nutrition of plants (26), which is the key factor of nodulation and nodule activities (19, 20).

These specialized tripartite symbioses among legumes, rhizobia and mycorrhizal fungi are maintained on complex source-sink relationships between each symbiont (8, 13, 14, 27, 31). Therefore, absence of an alternative symbiont sometimes reduces symbiotic effects entirely, and in some cases brings negative effects (18, 35). If this ineffectiveness results from nutritional imbalance, it can be recovered by an adequate fertilization. Several experiments were repeatedly conducted for functional replacement of symbionts with artificial fertilization (9, 13, 27), but a single effect of each symbiont had not been fully clarified yet.

Objectives of this study are to clarify whether a single inoculation of vesicular-arbuscular mycorrhizal (VAM) fungi induces marked growth enhancement of three *Acacia* species under a moderate nitrogen supply. In addition, this experiment was conducted in two soil water regimes (a moist and a dry condition) to determine influences of mycorrhiza on drought tolerance of legume trees.

## II. Materials and Methods

Nutrient-poor weathered granite excavated in Makabe district Ibaraki prefecture was used as a potting medium. The soil was sandy and its chemical property was as follows: pH (H<sub>2</sub>O) was 6.5, CEC was 8.4 meq 100 g<sup>-1</sup> dry soil, organic matter content was 0.02%, total nitrogen content was 0.01%, total phosphorus content was less than measurable value, water-soluble potassium content was 5 mg kg<sup>-1</sup> soil, exchangeable calcium and magnesium concentration were 821 and 71 mg kg<sup>-1</sup> soil, respectively. Soils were sieved through a 4 mm mesh stainless sieve to remove uncracked rocks, pebbles and plant roots. Sieved materials were steam-sterilized (100°C, 1 h) to eliminate native propagules of VA mycorrhizal fungi, then air-dried. The soil was filled into 1/10,000 a plastic pots. Whole weight of each pot was adjusted to the same value to control the soil water condition evenly. Before sowing, two grams of slow-release granulous fertilizer (N, P, K concentration was 16, 3 and 10 % in weight, respectively) were mixed into potting medium of each pot. The fertilizer is film-coated for releasing nutrients through three months.

Seeds of three *Acacia* species (*Acacia melanoxylon* R. BR., *Acacia mangium* WILLD., *Acacia auriculiformis* A. CUNN. ex. BENTH) were supplied from CSIRO Tree Seed Centre, Australia. Just before sowing, seeds were soaked in concentrated sulfuric acid for 20 min and washed several times with distilled water. This treatment effectively enhanced seed germination. At the time of sowing, half of the pots were inoculated with VA mycorrhizal fungal spores. Approximately 100 chlamyospores of *Glomus* spp. were poured into each four sowing holes in a pot. This inoculant was isolated from soils of a young sugi (*Cryptomeria japonica* D. DON) plantation in the Chiyoda Experimental Nursery of FFPRI by the wet sieving technique (21). The other half of the pots were inoculated with 20 ml of soil leachate containing no VAM fungal spores to minimize the other microbial effects. This leachate was attained by filtering soil suspension (mixture of 100 g fresh soils and one liter of distilled water) with a cheesecloth.

After the seed germination, seedlings were thinned to four per pot. Every pot was arranged in a greenhouse and irrigated well for a first month. To compensate for poor nutrient release from the granulous fertilizer at an earlier period, liquid fertilizer was added (50 ml per pot each time) to every pot weekly for the first month. Components of liquid fertilizer were as follows: 500 ppm nitrogen as NH<sub>4</sub>NO<sub>3</sub>, 200 ppm potassium as KCl, and 50 ppm magnesium as MgSO<sub>4</sub>. Other elements were not applied artificially.

One month from germination, all pots were translocated to growth chambers. Inner temperature of those chambers was adjusted as 30°C in the daytime and 25°C in the night. During a period in the chamber, irrigation of half of the pots was limited, and soil water potential was kept approximately less than -0.05 MPa as measured by a compact Tensiometer. Water supply was least limited in *A. mangium* and most

limited in *A. auriculiformis*, according to the rate of water loss from the pots. Because of sand-like characteristics of weathered granite, critical control of the water condition was formidable. Therefore, total weights of each pot were measured every day, and adjusted to a certain value with deionized water. Weights of the other pots were also measured every day and their water condition was kept adequate (soil water potential approximately ranged from  $-0.01$  to  $-0.04$  MPa) with deionized water. Accidentally withered seedlings were soon replanted with reserved seedlings with or without VAM. Every combination among tree species, inoculation and water control was replicated three times.

During a growth period, height and phyllode numbers of all seedlings were measured monthly until harvest. Three months after germination, all seedlings were harvested, checked for their natural nodulation, and measured for their height, phyllode numbers, root collar diameter, fresh and dry weight of shoot and root. To estimate VAM fungal colonization rate, some root segments were sampled, cleared with 10% aqueous KOH solution and stained with 0.1% trypan blue in lactophenol (36, 39). Stained roots were dispersed in Petri dish, placed under binocular dissecting microscope, and colonization rate was estimated by gridline intersect method (3). VAM fungal spores in potting medium were also counted after the experiment to examine the fungal reproduction. Spores were isolated from 100 g soil by wet sieving technique, and only fresh-looking spores were counted as newly formed spores.

Dried shoot and root samples were finely ground for nutrient analysis. Nitrogen concentration was determined with CN Corder (Yanaco MT-600). Due to poor biomass production of some seedlings (particularly seedlings in the dry condition), the nitrogen analysis could not be performed in some cases (see Fig. 3). After samples were digested with nitric acid-perchloric acid mixture, phosphorus concentration was determined colorimetrically by molybdenum blue method. Concentrations of Mg, Ca, Fe, Mn, Cu, and Zn were determined by atomic absorption spectrophotometry. Concentrations of K and Na were determined by flame photometer.

All the measured values were statistically calculated and compared by Duncan's new multiple range test to confirm significant differences between each treatment.

### III. Results

#### 1. VAM fungal colonization

Though colonization rate of VAM fungi varied from 1 to 80% among tree species and water conditions, every inoculated seedling formed VAM (Table 1). Among the three species, *A. auriculiformis* showed relatively lower colonization rate than the other two species. The dry treatment negatively affected colonization, particularly for *A. melanoxydon*: the dry treatment almost eliminated colonization in *A.*

Table 1. Final VAM infection rate of inoculated seedlings and VAM fungal spore numbers in a potting medium\*

Tree species	Treatment	Infection rate(%)	Spore numbers**
<i>Acacia melanoxydon</i>	moist	61.7 <sup>b</sup>	7.40 <sup>a</sup>
	dry	1.0 <sup>a</sup>	2.22 <sup>a</sup>
<i>Acacia mangium</i>	moist	80.7 <sup>b</sup>	4.67 <sup>a</sup>
	dry	41.0 <sup>a</sup>	4.44 <sup>a</sup>
<i>Acacia auriculiformis</i>	moist	35.7 <sup>b</sup>	3.18 <sup>a</sup>
	dry	26.3 <sup>a</sup>	2.45 <sup>a</sup>

\* Means of three replications. Means in a column followed by the same letter are not significantly different ( $p > 0.05$ ). Values are independent between tree species. No data of control seedlings are represented because they were VAM free. \*\* Values are calculated as numbers per gram of dry soil.

*melanoxydon*, and the colonization rate was less than 1%. Fungal reproduction accounted as newly developed extraradical spores was also depressed by dry treatment in *A. melanoxydon*. For the other two species, spore numbers were highly varied and did not differ significantly.

Non-inoculated seedlings had no VAM. Natural nodulation was not detected on any seedlings. This confirms the absence of infective propagules of rhizobium for these Acacias in experimental circumstances including applied soil leachate.

#### 2. Growth response

Patterns of growth responses for inoculation were clearly different among the three species (Fig. 1, Table 2).

Height growth and dry mass production of *A. melanoxydon* did not significantly differ between

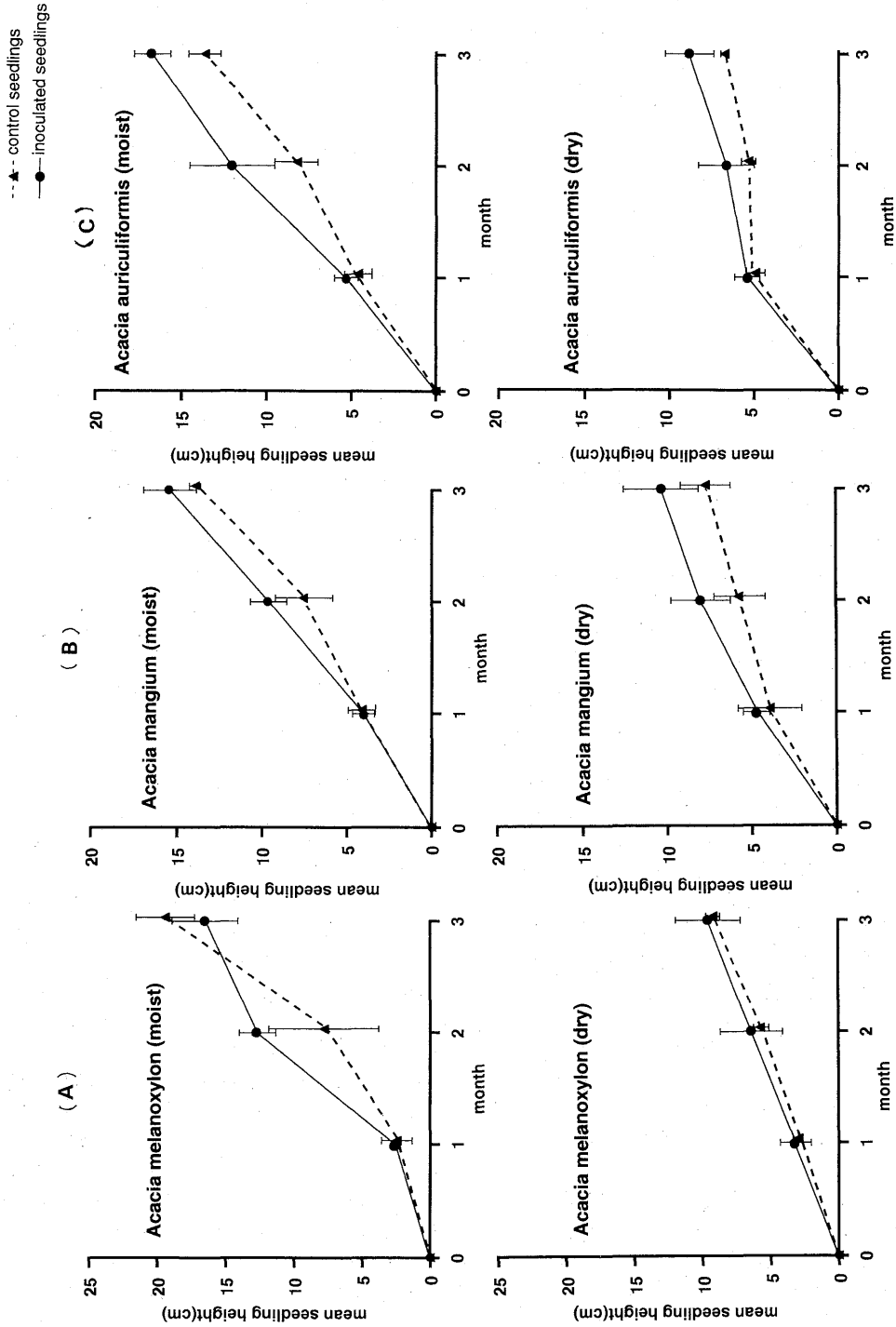


Fig. 1. Height growth of *Acacia melanoxylon* (A), *Acacia mangium* (B), and *Acacia auriculiformis* (C), seedlings. Vertical bar represents standard deviation.

inoculated seedlings and control. However, inoculated seedlings in the moist condition became somewhat dwarfed, which was characterized by a relatively short and thick trunk (see Table 2) bearing many axillary buds.

*A. mangium* showed a positive response for inoculation at the early growth stage. However, powdery mildew disease occurred and caused severe damage on whole leaves of inoculated plants, particularly those in the moist condition. The disease appeared slightly in control plants, and damage was negligible. As a result of the disease damage, heavy defoliation of inoculated plants nullified early stimulative effects of inoculation. Although the water supply was least limited for *A. mangium*, they wilted faster than the other two species, and withering occurred only in *A. mangium*. The mortality was higher in inoculated seedlings (seven seedlings withered in inoculated pots vs. two seedlings in control pots).

*A. auriculiformis* responded positively for inoculation both in the moist and the dry condition. Biomass production was significantly stimulated by VAM fungal inoculation, but differences were not extensive (magnitude was less than twice). Height growth did not differ significantly between inoculated and control seedlings over the whole experimental period. Effects of growth improvement were relatively greater in the dry condition, even though VAM colonization rate tended to be depressed by water deficit.

From those results, growth improvement derived from single inoculation of VAM fungi was slight or negligible compared with results of dual inoculation reported before (23, 33).

### 3. Nutrient status

Regardless of tree species and the water condition, phosphorus concentration in plant tissues was 25 to 145% higher in inoculated seedlings than control seedlings (Fig. 2). Differences were more evident in the moist condition than in the dry condition. Nitrogen concentration varied between 2.3 to 3.4% in shoots and 1.7 to 2.4% in roots. Specific tendency concerning inoculation or water conditions could not be detected (Fig. 3).

Mineral concentrations are summarized in Table 3a, b. In shoots, potassium concentration was relatively lower in inoculated *A. melanoxydon*, and higher in inoculated *A. mangium* and *A. auriculiformis* than control. Calcium concentrations showed trends opposite potassium. This tendency was significant in the moist condition, but not in the dry condition. In roots, potassium concentrations were lower in every inoculated seedling than control, while calcium concentrations were not affected by inoculation. Both in shoots and roots, total concentrations of major cations (K, Ca, Mg) plus sodium were not different significantly between inoculated and control seedlings regardless of water conditions.

Among minor elements, copper concentrations were consistently higher in inoculated seedlings both in

Table 2. Biomass, size and morphological features of harvested seedlings\*

Tree species	Treatment		Seedling height (cm)	Root collar diameter (mm)	Total phyllode numbers	Dry weight (g/seedling)		Root/shoot ratio	Height/RCD*** ratio
	VA**	Water				Shoot	Root		
<i>Acacia melanoxydon</i>	-	moist	19.4 <sup>b</sup>	3.04 <sup>b</sup>	13.5 <sup>b</sup>	4.63 <sup>b</sup>	1.89 <sup>b</sup>	0.40 <sup>a</sup>	6.51 <sup>b</sup>
	-	dry	9.2 <sup>a</sup>	2.01 <sup>a</sup>	9.9 <sup>a</sup>	1.67 <sup>a</sup>	0.71 <sup>a</sup>	0.42 <sup>a</sup>	4.57 <sup>a</sup>
	+	moist	16.5 <sup>b</sup>	4.30 <sup>c</sup>	12.6 <sup>b</sup>	4.86 <sup>b</sup>	2.29 <sup>b</sup>	0.47 <sup>a</sup>	3.84 <sup>a</sup>
	+	dry	9.6 <sup>a</sup>	2.20 <sup>a</sup>	10.3 <sup>a</sup>	1.64 <sup>a</sup>	0.65 <sup>a</sup>	0.40 <sup>a</sup>	4.30 <sup>a</sup>
<i>Acacia mangium</i>	-	moist	13.9 <sup>c</sup>	2.79 <sup>b</sup>	11.9 <sup>a</sup>	5.23 <sup>b</sup>	1.75 <sup>c</sup>	0.34 <sup>ab</sup>	5.03 <sup>a</sup>
	-	dry	7.7 <sup>a</sup>	1.89 <sup>a</sup>	9.6 <sup>a</sup>	1.39 <sup>a</sup>	0.54 <sup>a</sup>	0.52 <sup>b</sup>	4.14 <sup>a</sup>
	+	moist	15.4 <sup>c</sup>	2.65 <sup>b</sup>	15.2 <sup>b</sup>	4.15 <sup>b</sup>	1.13 <sup>b</sup>	0.26 <sup>a</sup>	5.86 <sup>a</sup>
	+	dry	10.3 <sup>b</sup>	1.81 <sup>a</sup>	10.5 <sup>a</sup>	2.00 <sup>a</sup>	0.83 <sup>b</sup>	0.42 <sup>b</sup>	5.74 <sup>a</sup>
<i>Acacia auriculiformis</i>	-	moist	13.6 <sup>c</sup>	3.35 <sup>b</sup>	12.0 <sup>c</sup>	4.84 <sup>c</sup>	2.24 <sup>c</sup>	0.46 <sup>a</sup>	4.05 <sup>a</sup>
	-	dry	6.7 <sup>a</sup>	1.62 <sup>a</sup>	6.4 <sup>a</sup>	0.79 <sup>a</sup>	0.43 <sup>a</sup>	0.55 <sup>a</sup>	4.16 <sup>a</sup>
	+	moist	16.7 <sup>d</sup>	3.82 <sup>b</sup>	13.8 <sup>d</sup>	5.93 <sup>d</sup>	2.79 <sup>d</sup>	0.47 <sup>a</sup>	4.40 <sup>a</sup>
	+	dry	8.8 <sup>b</sup>	1.95 <sup>a</sup>	7.9 <sup>b</sup>	1.54 <sup>b</sup>	0.78 <sup>b</sup>	0.51 <sup>a</sup>	4.51 <sup>a</sup>

\* Means of three replications. Means in a column followed by the same letter are not significantly different ( $p > 0.05$ ). Values are independent between tree species. \*\* - = Not inoculated (control), + = Inoculated. \*\*\* RCD = Root collar diameter.

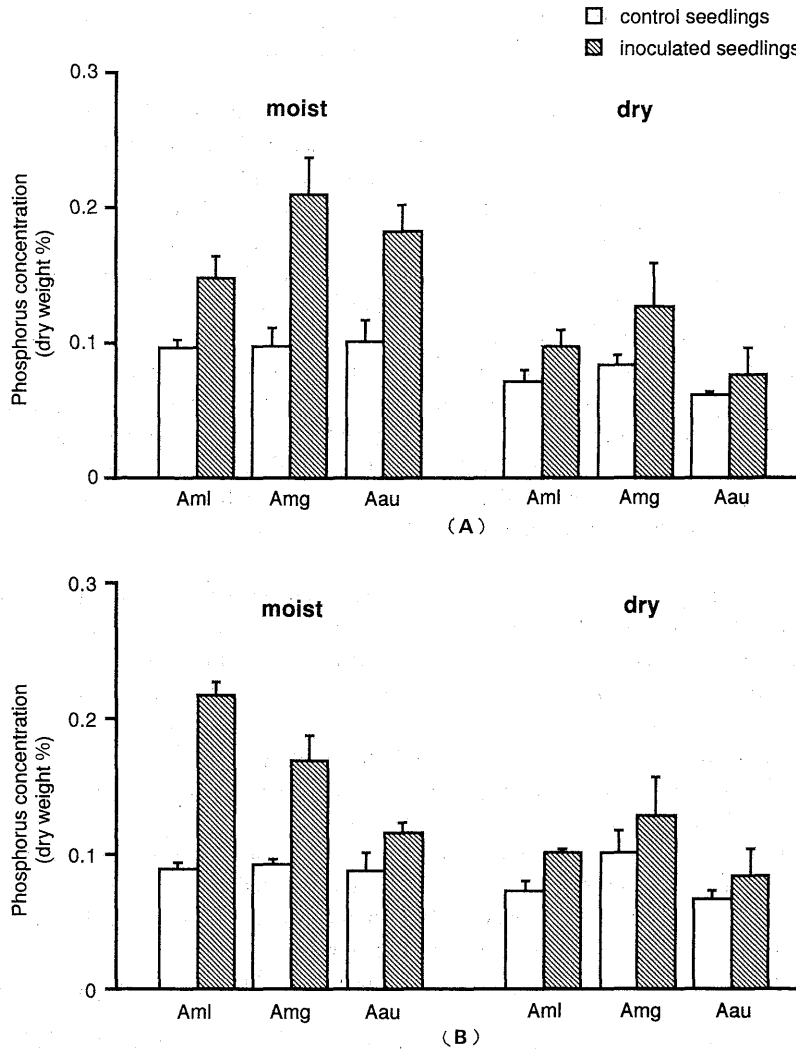


Fig. 2. Phosphorus concentrations in seedling shoots (A) and roots (B)

Vertical bar represents standard deviation. Aml=*A. melanoxylon*, Amg=*A. mangium*, Aau=*A. auriculiformis*.

shoots and roots. Improved absorption of zinc by VAM was often reported (5, 22), but marked influence of inoculation on zinc nutrition was not exhibited except in shoots of *A. mangium*. For other elements, distinctive differences between inoculated and control seedlings were not detected.

#### IV. Discussion

##### 1. Growth responses for VAM fungal inoculation

Though nitrogen fertilizer was supplied instead of biosynthetic nitrogen supply, effect of VA mycorrhizal inoculation was quite slight. The most conceivable reason for this poor response is still nitrogen deficiency. Total amount of nitrogen that had been released and accessible for absorption during the experimental period was expected to be approximately 100 mg/seedling. Although this amount was calculated from a nutrient analysis of several leguminous trees (AKAMA, personal communication), growth rate

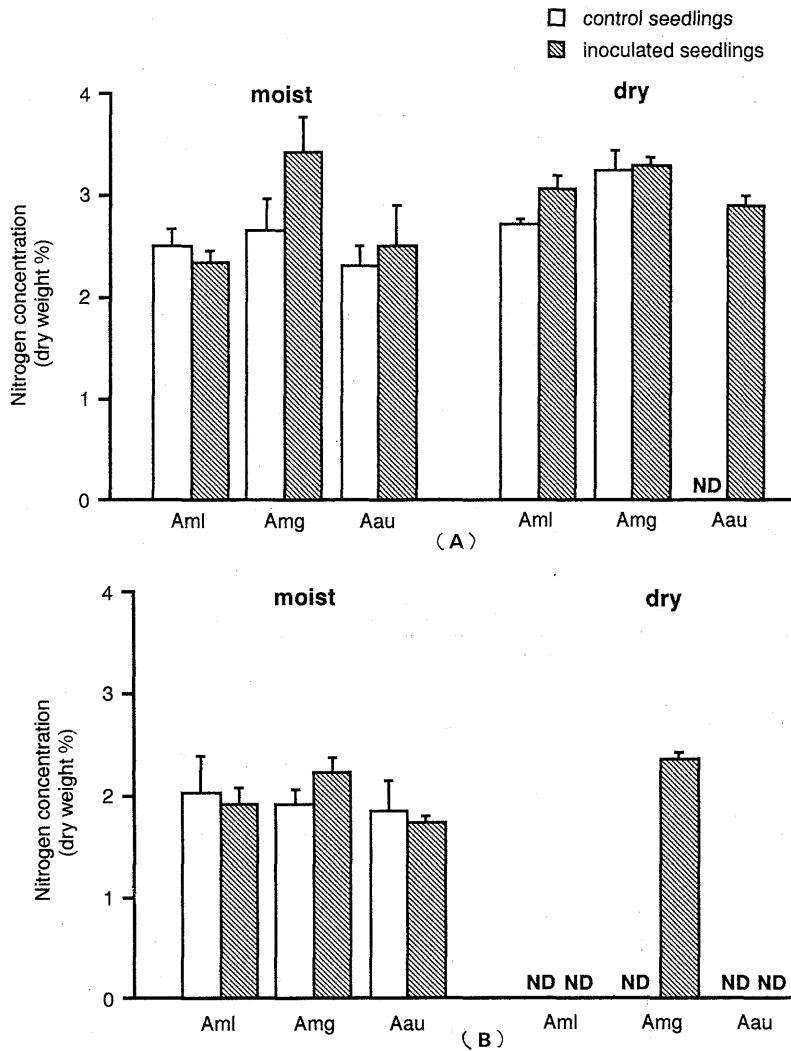


Fig. 3. Nitrogen concentrations in seedling shoots (A) and roots (B)  
 Vertical bar represents standard deviation. ND=not determined. Aml=*A. melanoxylon*, Amg=*A. mangium*, Aau=*A. auriculiformis*.

and nitrogen concentration in shoots were relatively lower than other reported values of legume trees (4, 23, 33). It was not clear why the applied nitrogen was insufficient for maintaining adequate growth, but some reasons can be pointed out. Nearly 90% of applied nitrogen was supplied as slow-release form. Hence, some amounts of nitrogen may have been still unreleased at the end of the experiment. This insufficient nitrogen supply may act as a strong growth-limiting factor, particularly at earlier stage of seedling growth. Extensive seedling numbers per pot was also a possible cause of inhibiting fair nitrogen nutrition during a growth period.

However, even though nitrogen starvation inhibited sufficient growth, it is aberrant why any evident growth stimulation was not observed regardless of mycorrhizal activity accounted as phosphorus nutrition. It seems that additional limiting factors also affected on seedling growth simultaneously with nitrogen shortage.

VA mycorrhizal fungi themselves may have negative effect on seedling growth. Benefit from mycorrhizal



Table 3-a. Mineral concentrations in seedling shoots\*

Tree species	Treatment		K (%)	Ca (%)	Mg (%)	Na (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
	VA**	Water								
<i>Acacia melanoxylon</i>	-	moist	0.94 <sup>b</sup>	0.68 <sup>a</sup>	0.13 <sup>b</sup>	0.07 <sup>a</sup>	70 <sup>a</sup>	290 <sup>a</sup>	13 <sup>b</sup>	2 <sup>a</sup>
	-	dry	0.81 <sup>a</sup>	0.70 <sup>a</sup>	0.11 <sup>a</sup>	0.05 <sup>a</sup>	65 <sup>a</sup>	232 <sup>a</sup>	10 <sup>a</sup>	2 <sup>a</sup>
	+	moist	0.71 <sup>a</sup>	0.78 <sup>a</sup>	0.16 <sup>c</sup>	0.05 <sup>a</sup>	113 <sup>b</sup>	370 <sup>a</sup>	11 <sup>ab</sup>	5 <sup>b</sup>
	+	dry	0.78 <sup>a</sup>	0.74 <sup>a</sup>	0.13 <sup>b</sup>	0.08 <sup>a</sup>	90 <sup>ab</sup>	291 <sup>a</sup>	10 <sup>a</sup>	5 <sup>b</sup>
<i>Acacia mangium</i>	-	moist	0.95 <sup>a</sup>	0.87 <sup>ab</sup>	0.13 <sup>a</sup>	0.08 <sup>a</sup>	93 <sup>a</sup>	181 <sup>a</sup>	11 <sup>a</sup>	1 <sup>a</sup>
	-	dry	0.72 <sup>a</sup>	0.74 <sup>a</sup>	0.13 <sup>a</sup>	0.13 <sup>a</sup>	120 <sup>ab</sup>	240 <sup>a</sup>	15 <sup>a</sup>	1 <sup>a</sup>
	+	moist	1.52 <sup>b</sup>	0.74 <sup>a</sup>	0.18 <sup>b</sup>	0.12 <sup>a</sup>	104 <sup>a</sup>	155 <sup>a</sup>	23 <sup>b</sup>	6 <sup>b</sup>
	+	dry	0.81 <sup>a</sup>	0.95 <sup>b</sup>	0.14 <sup>a</sup>	0.12 <sup>a</sup>	164 <sup>b</sup>	209 <sup>a</sup>	17 <sup>a</sup>	3 <sup>b</sup>
<i>Acacia auriculiformis</i>	-	moist	0.87 <sup>b</sup>	1.02 <sup>c</sup>	0.16 <sup>a</sup>	0.07 <sup>a</sup>	80 <sup>a</sup>	103 <sup>ab</sup>	15 <sup>a</sup>	1 <sup>a</sup>
	-	dry	0.74 <sup>a</sup>	0.70 <sup>a</sup>	0.15 <sup>a</sup>	0.11 <sup>a</sup>	74 <sup>a</sup>	118 <sup>b</sup>	13 <sup>a</sup>	4 <sup>ab</sup>
	+	moist	0.94 <sup>c</sup>	0.90 <sup>b</sup>	0.17 <sup>a</sup>	0.08 <sup>a</sup>	69 <sup>a</sup>	90 <sup>a</sup>	17 <sup>a</sup>	6 <sup>b</sup>
	+	dry	0.78 <sup>a</sup>	0.86 <sup>b</sup>	0.14 <sup>a</sup>	0.10 <sup>a</sup>	75 <sup>a</sup>	102 <sup>ab</sup>	16 <sup>a</sup>	4 <sup>ab</sup>

\* Means of three replications. Values are calculated as dry weight bases. Means in a column followed by the same letter are not significantly different ( $p > 0.05$ ). Values are independent between tree species. \*\* - = not inoculated (control), + = inoculated.

Table 3-b. Mineral concentrations in seedling roots\*

Tree species	Treatment		K (%)	Ca (%)	Mg (%)	Na (%)	Fe (%)	Mn (%)	Zn (%)	Cu (ppm)
	VA**	Water								
<i>Acacia melanoxylon</i>	-	moist	2.27 <sup>c</sup>	0.39 <sup>b</sup>	0.43 <sup>b</sup>	0.37 <sup>a</sup>	0.54 <sup>a</sup>	0.14 <sup>b</sup>	0.041 <sup>c</sup>	10 <sup>a</sup>
	-	dry	1.32 <sup>a</sup>	0.37 <sup>ab</sup>	0.32 <sup>ab</sup>	0.52 <sup>b</sup>	0.83 <sup>b</sup>	0.06 <sup>a</sup>	0.031 <sup>bc</sup>	11 <sup>a</sup>
	+	moist	1.93 <sup>b</sup>	0.37 <sup>ab</sup>	0.50 <sup>b</sup>	0.38 <sup>a</sup>	0.89 <sup>b</sup>	0.13 <sup>b</sup>	0.020 <sup>ab</sup>	16 <sup>b</sup>
	+	dry	1.37 <sup>a</sup>	0.29 <sup>a</sup>	0.24 <sup>a</sup>	0.50 <sup>b</sup>	0.65 <sup>a</sup>	0.08 <sup>a</sup>	0.013 <sup>a</sup>	15 <sup>b</sup>
<i>Acacia mangium</i>	-	moist	1.88 <sup>b</sup>	0.44 <sup>b</sup>	0.37 <sup>b</sup>	0.20 <sup>a</sup>	0.63 <sup>a</sup>	0.14 <sup>b</sup>	0.036 <sup>c</sup>	12 <sup>a</sup>
	-	dry	1.49 <sup>a</sup>	0.27 <sup>a</sup>	0.18 <sup>a</sup>	0.25 <sup>a</sup>	0.63 <sup>a</sup>	0.07 <sup>a</sup>	0.011 <sup>ab</sup>	13 <sup>a</sup>
	+	moist	1.43 <sup>a</sup>	0.42 <sup>b</sup>	0.40 <sup>b</sup>	0.23 <sup>a</sup>	0.81 <sup>a</sup>	0.13 <sup>b</sup>	0.016 <sup>b</sup>	24 <sup>b</sup>
	+	dry	1.23 <sup>a</sup>	0.29 <sup>a</sup>	0.21 <sup>a</sup>	0.22 <sup>a</sup>	0.86 <sup>a</sup>	0.06 <sup>a</sup>	0.006 <sup>a</sup>	16 <sup>a</sup>
<i>Acacia auriculiformis</i>	-	moist	1.42 <sup>d</sup>	0.53 <sup>b</sup>	0.24 <sup>b</sup>	0.17 <sup>b</sup>	0.49 <sup>a</sup>	0.12 <sup>c</sup>	0.011 <sup>b</sup>	9 <sup>a</sup>
	-	dry	0.72 <sup>a</sup>	0.36 <sup>a</sup>	0.15 <sup>a</sup>	0.12 <sup>a</sup>	0.71 <sup>a</sup>	0.05 <sup>a</sup>	0.007 <sup>a</sup>	11 <sup>a</sup>
	+	moist	1.13 <sup>c</sup>	0.48 <sup>b</sup>	0.24 <sup>b</sup>	0.15 <sup>ab</sup>	0.67 <sup>a</sup>	0.08 <sup>b</sup>	0.010 <sup>ab</sup>	13 <sup>a</sup>
	+	dry	0.90 <sup>b</sup>	0.37 <sup>a</sup>	0.18 <sup>ab</sup>	0.21 <sup>c</sup>	0.72 <sup>a</sup>	0.05 <sup>a</sup>	0.007 <sup>a</sup>	12 <sup>a</sup>

\* Means of three replications. Values are calculated as dry weight bases. Means in a column followed by the same letter are not significantly different ( $p > 0.05$ ). Values are independent between tree species. \*\* - = not inoculated (control), + = inoculated.

symbiosis is mainly attained from nutritional improvement (1). It has been confirmed that if this nutritional improvement by mycorrhiza has minute on host's growth and photosynthetic activity, mycorrhizal fungus often behaves as a simple carbon sink for a host, the same as a parasitic fungus (10, 11, 29, 30). Calculated carbon cost of VAM fungi was varied from 4% to 17% of total photosynthate of host plants (27, 31). Those rates seem to be enough to inhibit growth of stressed plants. Therefore, benefit of mycorrhizal inoculation may be withheld by carbon loss from the hosts in this experiment.

Nutrient imbalance often affects physiological activity of host plants and negates the effect of inoculation; e. g. factors such as phosphorus/nitrogen ratio may affect seedling growth. Furthermore, it was reported that externally applied nitrogen was ineffective on some fatty acid synthesis of soybean compared with biosynthetic supplied nitrogen (34). Hence, it is suggested that nitrogen metabolic pathways are different between external and biosynthetic nitrogen, and they may affect seedling growth. However, more examination is necessary to confirm it.

## 2. Effect of VAM fungal inoculation on drought stress

Growth of seedling grown under dry conditions was somewhat ameliorated by inoculation. However, the degree of its effect was varied among tree species. Although water supply was least limited in *A. mangium*, the influence of drought stress was the most strong. It seems that large phyllodes of *A. mangium* transpired more vigorously, and this induced faster desiccation. Furthermore, defoliation and withering caused by drought stress appeared earlier and heavier in inoculated seedlings than control. HARDIE and LEYTON determined water relations of red clover inoculated with a VAM fungus, and showed that inoculated plants had higher transpiration rate, higher root hydraulic conductivity and lower stomatal resistance (25). Those results suggest that VAM enhances water transportation of host plants. Under the slight drought stress, VAM aids the water uptake of host plants. However, under severer drought stress, VAM depletes root zone water faster than non-mycorrhizal root, then water deficit appears faster and severer in VAM plants (25, 28, 32).

Among the three *Acacia* species, growth differences between inoculated and control seedlings in the dry condition were most evident for *A. auriculiformis*. Superficially, this seems attributable to the effect of VAM inoculation. However, this growth difference may simply reflect a level of stored nutrient or a preserved difference of biomass before drought, because VAM colonization decreased and numerous root hairs formed in the dry condition. Recently, OSONUBI *et al.* reported that VAM fungal inoculation did not improve growth and nutrition of *A. auriculiformis* seedlings in a dry condition. In contrast, inoculation of an ectomycorrhizal fungus extremely improved them (33). Contribution of VAM in a dry condition seems strongly related to host-fungus adaptability and drought tolerance of mycorrhizal fungi, and must be considered more critically.

VAM colonization rate and fungal reproduction were somewhat decreased by the dry treatment. The same phenomenon has been reported, though the magnitude was varied by drought severity, host and fungal species (12, 15). Different degree of depressed infection by drought among three host tree species is partly derived from difference of VAM fungal activity and root growth during the drought period. Therefore, for application of those VAM fungi to an area that has regularly drought, selection of suitable fungi that are not only effective for tree growth but also to colonize and reproduce under such conditions is important to obtain maximum and stable effect of mycorrhiza.

## 3. Other influences from VAM fungal inoculation

It is known that absorption of highly mobile nutrients such as K and Ca were slightly affected by VAM (26). However, K/Ca ratio was different between inoculated and control seedlings, even though total concentrations of major cations were nearly equal. Different from phosphorus, selective uptake and transport systems of highly mobile ions may be altered by host physiological status, not directly affected by VAM.

In this experiments, severe powdery mildew disease occurred particularly on inoculated seedlings. There were several reports about disease suppression by VAM fungal inoculation (summarized by DEHNE (17)). However, such effects of VAM were affirmed only for root disease, and meager or negative effects were reported on shoot (leaf) disease (*e. g.* *Verticilium* wilt of cotton (16)). It is not obvious why VAM fungal inoculation accelerated disease, but phosphorus or some nutritional changes may induce disease severity.

Inoculated seedlings of *A. melanoxylon* showed different morphological features compared with control seedlings. One of the well-known morphological changes induced by VAM is decrease of root/shoot ratio (24, 38), but such changes were not evident in this case. It is known that VAM affect phytohormone mediation of host plants directly through their own hormone production or indirectly through VAM-mediated phosphorus or other nutrients nutrition (2, 6). Stressed condition of this experiment may induce not only nutritional imbalance but also hormonal (*e. g.* cytokinin) imbalance by VAM.

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(Received March 16, 1992)