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Effects of ectomycorrhizal and vesicular-arbuscular mycorrhizal fungi on drought tolerance of four leguminous woody seedlings

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Abstract

Seedlings of *Acacia auriculiformis* A. Cunn. ex. Benth., *Albizia lebbeck* (L.) Benth., *Gliricidia sepium* (Jac.) Walp and *Leucaena leucocephala* (Lam.) de Wit. were inoculated with an ectomycorrhizal (*Boletus suillus* (L. ex. Fr.) or indigenous vesicular-arbuscular mycorrhizal (VAM) fungi in a low P soil. The plants were subjected to unstressed (well-watered) and drought-stressed (water-stressed) conditions. In *Gliricidia* and *Leucaena*, both mycorrhizal inoculations stimulated greater plant growth, P and N uptake compared to their non-mycorrhizal (NM) plants under both watering regimes. However, in *Acacia* and *Albizia*, these parameters were only stimulated by either ectomycorrhiza (*Acacia*) or VA mycorrhiza (*Albizia*). Growth reduction occurred as a result of inoculation with the other type of mycorrhiza. This was attributed to competition for carbon between *Acacia* and VA mycorrhizas and parasitic association between *Albizia* and ectomycorrhiza.

Drought-stressed mycorrhizal and NM *Leucaena*, and drought-stressed mycorrhizal *Acacia* tolerated lower xylem pressure potentials and larger water losses than the drought-stressed mycorrhizal and NM *Albizia* and *Gliricidia*. These latter plants avoided drought by maintaining higher xylem pressure potentials and leaf relative water content (RWC). All the four leguminous plants were mycorrhizal dependent. The higher the mycorrhizal dependency (MD), the lower the drought tolerance expressed in terms of drought response index (DRI). The DRI may be a useful determinant of MD, as they are inversely related.

Introduction

The concept of using N-fixing woody legumes in enriching poor soils of the humid and subhumid tropics through a farming practice described as 'alley farming' is well known (Kang and Wilson, 1987). However, nodulation and subsequent N-fixation by rhizobia of leguminous plants require optimum level of P in host tissues (Hayman, 1986; Marschner, 1986). The vast areas of the humid and subhumid tropics are dominated by low activity clay soils which are low in available P and other nutrients (Kang and Wilson, 1987; Mulongoy et al., 1988) and they also suffer drought. Although plant species have evolved different adaptations for coping with these resource limitations (Kozlowski, 1982), additional contributions through mycorrhizal infection may be particularly useful.

Both ectomycorrhizal and vesicular-arbuscular mycorrhizal (VAM) fungi are known to increase nutrient uptake, particularly P, and biomass accumulation of many land plant species in low P soil (Ekwebelam and Reid, 1983; Mejstrik, 1975;

Nelsen and Safir, 1982). Furthermore, mycorrhizal fungi can increase the drought tolerance of host plants (Allen and Allen, 1986; Hardie and Leyton, 1981; Levy and Krikun, 1980; Osonubi, 1989). Where the growth of mycorrhizal plants was greater than NM plants, the increased growth was attributed to increased stomatal conductance (Allen and Boosalis, 1983; Osonubi, 1989; Parke et al., 1983) and root conductivity (Graham and Syvertsen, 1984) provided by increased surface area of mycorrhizal hyphae (Allen, 1982; Hardie and Leyton, 1981; see also reviews in Harley and Smith, 1983; Read and Boyd, 1986). These reported changes could be secondary responses to better P nutrition (Graham and Syvertsen, 1984, 1985; Michelsen and Rosendahl, 1990; Nelsen and Safir, 1982; Osonubi et al., 1990) or mediated via direct mycorrhizal effects (Henderson and Davies, 1990).

On the other hand, mycorrhizal infection has been shown to depress plant growth in soils with optimum P availability (Buwalda and Goh, 1982; Crush, 1976) or low P availability (Bethlenfalvay et al., 1982; Koide, 1985). These growth depressions have been attributed to competition for carbon between the host plant and the mycorrhizal fungus (Buwalda and Goh, 1982). Although, low availability of P in soil solution often creates growth limiting conditions for plants, its uptake may be increased by more efficient mycorrhizas (Smith and Gianinazzi-Pearson, 1988).

The purpose of this study was to evaluate the influence of both ecto- and VA mycorrhizas on the growth, nutrient uptake and drought tolerance of the four tropical tree legumes *Acacia auriculiformis*, *Albizia lebbeck*, *Gliricidia sepium* and *Leucaena leucocephala* and to assess the efficiencies of the mycorrhizas with respect to the above objectives under well-watered and drought-stressed conditions.

Materials and methods

Seedling preparation and inoculation

Seeds of *Acacia auriculiformis* A. Cunn. ex. Benth., *Albizia lebbeck* (L.) Benth., and *Leucaena leucocephala* (Lam.) de Wit. were scarified with concentrated H_2SO_4 for 30 minutes and washed several times in distilled water. Seeds of *Gliricidia* were surface sterilized in a 0.1% mercuric chloride for 3 minutes and then washed several times in distilled water. All the seeds were then inoculated with a peat base inoculum of *Rhizobium* strains isolated from each woody species. The seeds were germinated in plastic trays containing either sterilized sandy loam alone (non-mycorrhizal) or sandy loam inoculated with indigenous VAM roots of *Leucaena*. Each mycorrhizal tray was inoculated with 20 g of VAM roots. The VAM fungi mixture consists of two *Glomus* and one *Acaulospora* species (identification by courtesy of Dr S M Berch, University of British Columbia, Canada).

After 4 weeks of growth, ten seedlings of each species were transplanted from the VAM inoculated trays into black polyethylene bags (11.5 cm diameter, 32 cm deep) containing 2.5 kg sterilized sandy loam soil. The soil contained 4.82 mg kg^{-1} extractable P (Bray-1) 2.3 g kg^{-1} total N with a pH(H_2O) of 6.6.

Twenty seedlings of each species from the non-mycorrhizal trays were also transplanted into similar black polyethylene bags containing similar quantity of sandy loam soil after 4 weeks of growth. Half of these were inoculated with ectomycorrhizal fungus of *Boletus suillus* (L. ex Fr.) obtained from Drs C Leyval and J Berthelin of Vandoeuvre in France. The remaining plants were treated as non-mycorrhizal (NM) plants. Seedlings were inoculated at transplanting by pipetting 2 mL of the prepared ectomycorrhizal inoculum (Pachlewski, 1967) on the roots of each seedling at 3 cm below the soil surface. Afterwards the inoculation hole was covered with soil. Each of the NM seedlings remained uninoculated.

All seedlings were grown under well-watered conditions for another 8 weeks in the greenhouse under a maximum photosynthetic active radiation (PAR) of 1500 μmol m^{-2} s^{-1} and average day/night temperature of 35/25 ± 2°C before the commencement of experimental treatments. Six seedlings of each species from ectomycorrhizal, VAM and NM plants were randomly chosen for the experimental treatments. The experimental set up was a completely randomized design with

three replications and factorial treatments. One factor was the watering regime (i.e. unstressed or drought-stressed) and second factor was mycorrhizal treatment. The unstressed plants, mycorrhizal or NM, were watered daily, while the drought-stressed plants were watered to drip point (field capacity) at weekly intervals for another 12 weeks.

Physiological and growth measurements

After 12 cycles of drought-stress, i.e. 24 weeks after germination, measurements were made of xylem pressure potential, leaf relative water content (RWC), soil moisture content, leaf area, root length, shoot and root dry weights. Xylem pressure potential and RWC measurements were made between 1200 and 1400 h because preliminary experiments showed that this time was the period of maximum stress. Xylem pressure potential was determined with a pressure chamber apparatus (Soil Moisture Instruments Co., Santa Barbara, USA) using single shoots of the plants. Leaf area was determined with a LiCor Li-3000 Planimeter (LiCor Inc., Lincoln, Nebraska). Relative water content was determined using the modified method of Hewlett and Kramer (1963) and calculated according to Kramer and Kozlowski (1979). Midveins of leaflets in *Acacia* and *Gliricidia* were removed after detaching them from the plants. While leaflets of *Albizia* and *Leucaena* were used because of their relatively small midveins. Fresh weights of these leaflets were taken and then floated on distilled water at room temperature for 4 hours. They were superficially dried between the filter papers and weighed to obtain the turgid weight. Dry weights were determined after oven drying at 80°C for one day.

Soil moisture content was determined by retrieving the soil column from the polyethylene bag. A soil sample was taken from midway between the soil surface and root growth limit as being representative of the soil moisture available to the roots in each column. The sample was dried in the oven for a day at 80°C and soil moisture as a percentage of oven dry weight was calculated. Soil moisture content at field capacity was 24 ± 2% and at permanent wilting point was 5.2%.

Root length was determined by a line-intersect method (Tennant, 1975). Each soil column was soaked in 1% tetrasodium pyrophosphate solution for one day and the roots recovered by washing out the loose soil over a fine sieve.

P and N determination

Total plant dry weight was obtained by oven-drying the leaves, stems and roots separately for a day at 70°C. Plant shoot total N was determined colorimetrically using a Technicon autoanalyser (IITA Manual series, 1982) – after micro-Kjeldahl digestion of the samples with a mixture of selenium and concentrated H_2SO_4. Phosphorus concentration was determined by the molybdenum blue method (IITA manual series, 1982) after wet ashing the shoot samples in nitric-perchloric acid mixture (2:1 v/v), using a B and L Spectronic-70-Electrophotocolorimeter. The nutrient uptake was calculated by multiplying the percentage nutrient concentration with their corresponding shoot dry weights.

Mycorrhizal infection

Ectomycorrhizal infection was determined by cutting 1 cm root segments at 3 cm intervals down the whole root system. The 1 cm root segments for each root system were pooled and a quarter of the segments were randomly selected for evaluation of mycorrhizal infection under a dissecting microscope (Daughtridge et al., 1986; Gibson et al., 1988). Each lateral root on every selected quarter was scored for infection if microscopic examination revealed the presence of radiating hyphae. The percentage infection was expressed as the total number of infected laterals divided by the total number of laterals in the observed quarter multiplied by 100 (Daughtridge et al., 1986). Three replicates per treatment were examined in this manner. Under microscopic examinations, the mantle sheath on lateral roots was absent except for radiating hyphae, but Hartig nets separating cortical cells were observed using a compound microscope.

The VAM infection was determined by staining and assessing 1 cm root segments for presence or absence of arbuscles and vesicles using the magnified intersections method (Mcgonigle et al., 1990). On this basis, the infection was

expressed as percentage of the root infected. Root segments were preserved in formalin acetic acid-alcohol (FAA) solution until required. The root samples of each woody species were first cleared for 15 min at 121°C in 10% (w/v) KOH and washed several times in distilled water. The root samples were later stained in a 0.02% (w/v) Chlorazol black E solution made up in 80% lactic acid, glycerin and distilled water in equal volumes (1:1:1, v/v/v) (Brundrett et al., 1984), after bleaching the pigmented roots of *Acacia* and *Leucaena* with alkaline H_2O_2. Root segments were scored for VAM infection if the hyphae possessed arbuscles or vesicles.

Mycorrhizal dependency (MD) and drought response index (DRI)

Mycorrhizal dependency (MD) was expressed as the ratio of total dry weight of mycorrhizal plant and non-mycorrhizal plant (Graham and Syvertsen, 1985; Menge et al., 1978). Drought response index (DRI) was calculated as the ratio of total dry weight of mycorrhizal plant under drought stress to mycorrhizal plant under well-watered conditions.

Statistical analysis

The data for unstressed and drought-stressed treatments of ectomycorrhizal, VAM and NM plants of each species were subjected to a combined analysis of variance. Duncan's multiple range test and least significant difference at $p < 0.05$ were used to separate the means. The relationship between growth characteristics and nutrient uptake were evaluated using linear regression analysis.

Results

Mycorrhizal infection

Under well-watered conditions (24 weeks of daily watering) there were significant differences between species in development of both ectomycorrhizal and VAM infection (Table 1). The imposition of drought stress after infection had become established (12 weeks daily watering/12 weeks watering at weekly intervals) had no effect on the development of ectomycorrhizas in *Acacia*, *Albizia* or *Leucaena*, but a significant reduction was observed in *Gliricidia*. In contrast, VAM infection was lower in all the species, except for *Acacia* at the end of the period of drought stress.

Plant growth responses

Under unstressed conditions, *Gliricidia* and *Leucaena* had higher leaf area (Fig. 1), and both shoot and root dry weights (Fig. 2) when inoculated with both ectomycorrhizal and VAM fungi. *Acacia* showed a positive response to ectomycorrhizal infection, while the converse was true for *Albizia*. VAM and ectomycorrhizal inoculations significantly reduced the growth parameters of *Acacia* and *Albizia* respectively below their NM plants. In spite of the significant differences

Table 1. Percentage root infection of unstressed and drought-stressed ectomycorrhizal (ECTO) and vesicular-arbuscular mycorrhizal (VAM) plants at the end of experiment

	ECTO		VAM	
	Unstressed	Drought-stressed	Unstressed	Drought-stressed
Acacia auriculiformis	55.6b	45.6a	24.6d	23.0c
Albizia lebbeck	30.0d	24.0c	44.6c	34.4b*
Gliricidia sepium	66.7a	40.3b*	57.5b	39.4a*
Leucaena leucocephala	50.3c	41.6ab	68.4a	35.2b*

Means on the same vertical column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test. Also, means followed by * indicate significant difference at $p < 0.05$ between unstressed and drought-stressed plants of the same species. NM plants were never observed to be infected.

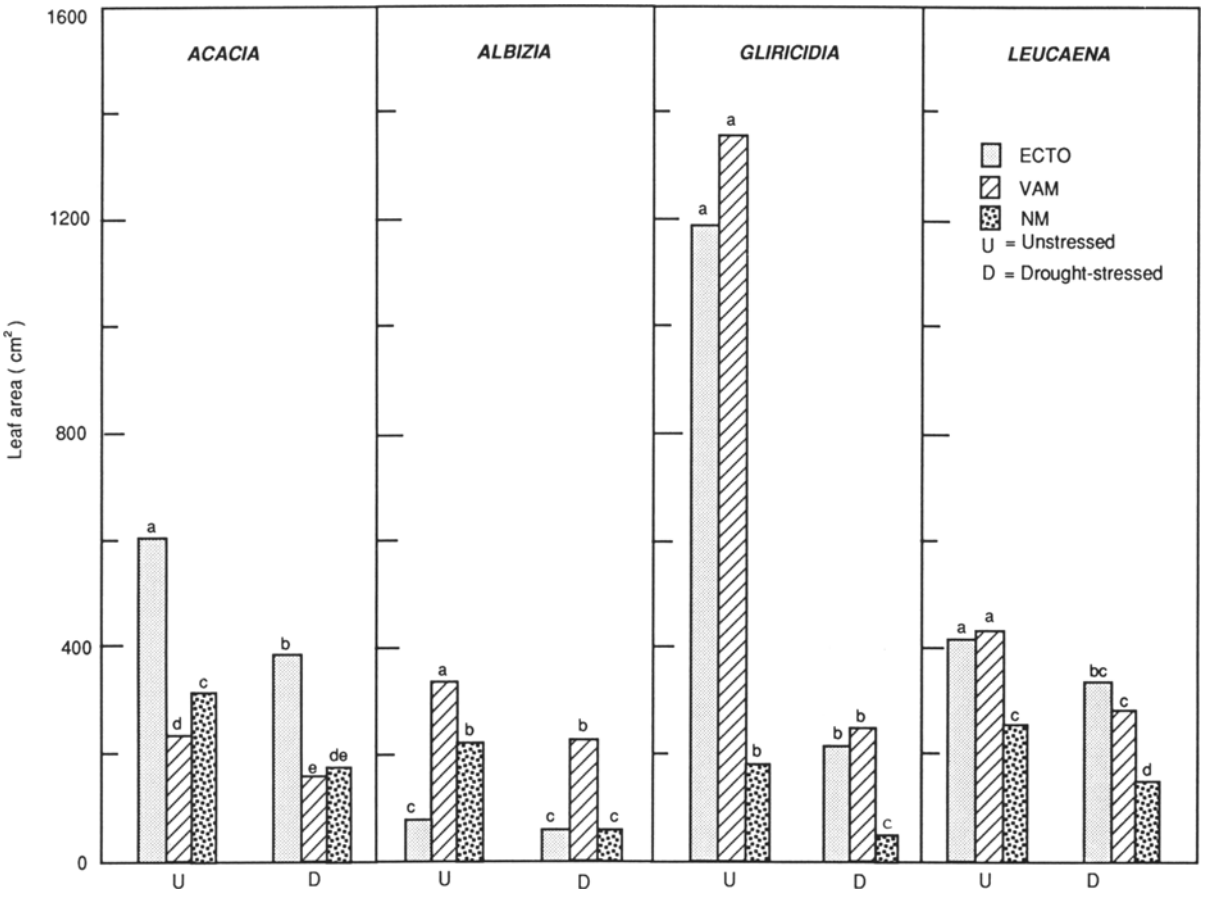

Fig. 1. Leaf area of unstressed (U) and drought-stressed (D) ectomycorrhizal (ECTO), vesicular-arbuscular mycorrhizal (VAM) and non-mycorrhizal (NM) of four leguminous woody seedlings at the end of experiment. *Bars* without the same letter above are significantly different at $p < 0.05$ according to Duncan's multiple range test.

($p < 0.05$) in leaf area between *Gliricidia* and *Leucaena*, both mycorrhizas caused comparable shoot and root biomass accumulations in both plants. In general, root length (Fig. 3) showed the same pattern of differences as the other parameters of growth, although the effect was not significant in *Leucaena*.

Drought stress significantly reduced the growth parameters below those of unstressed plants (Figs. 1, 2, and 3). Despite the non-significant difference between the leaf areas of mycorrhizal *Gliricidia* and *Leucaena* plants, both mycorrhizas caused significantly less ($p < 0.05$) biomass accumulation in shoots of *Gliricidia*, suggesting greater drought tolerance in *Leucaena*. There was no effect of drought stress on root length of mycorrhizal plants of *Leucaena* (Fig. 3).

P and N uptake

These showed the same pattern as the growth responses (Fig. 4). Unstressed *Gliricidia* and *Leucaena* had greater P uptake with VAM inoculation than ectomycorrhizal inoculation. Mycorrhizal *Gliricidia* had the highest P (VAM) and N uptake under unstressed conditions, whereas mycorrhizal *Leucaena* had the highest P and N uptake under drought-stressed conditions and these were not significantly different from the uptake in well-watered plants (Fig. 4).

Since the correlation coefficients between

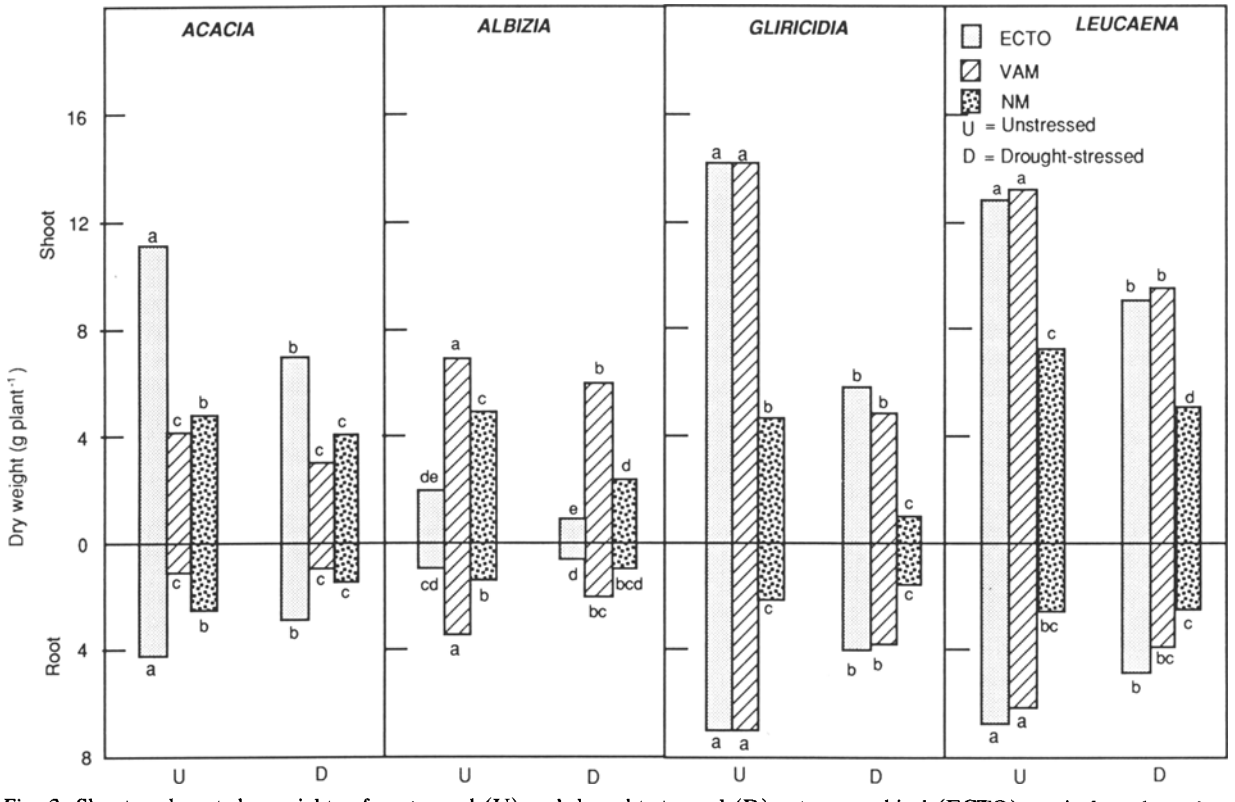

Fig. 2. Shoot and root dry weights of unstressed (U) and drought-stressed (D) ectomycorrhizal (ECTO), vesicular-arbuscular mycorrhizal (VAM) and non-mycorrhizal (NM) of four leguminous woody seedlings at the end of experiment. *Bars* without the same letter above or below are significantly different at $p < 0.05$ according to Duncan's multiple range test.

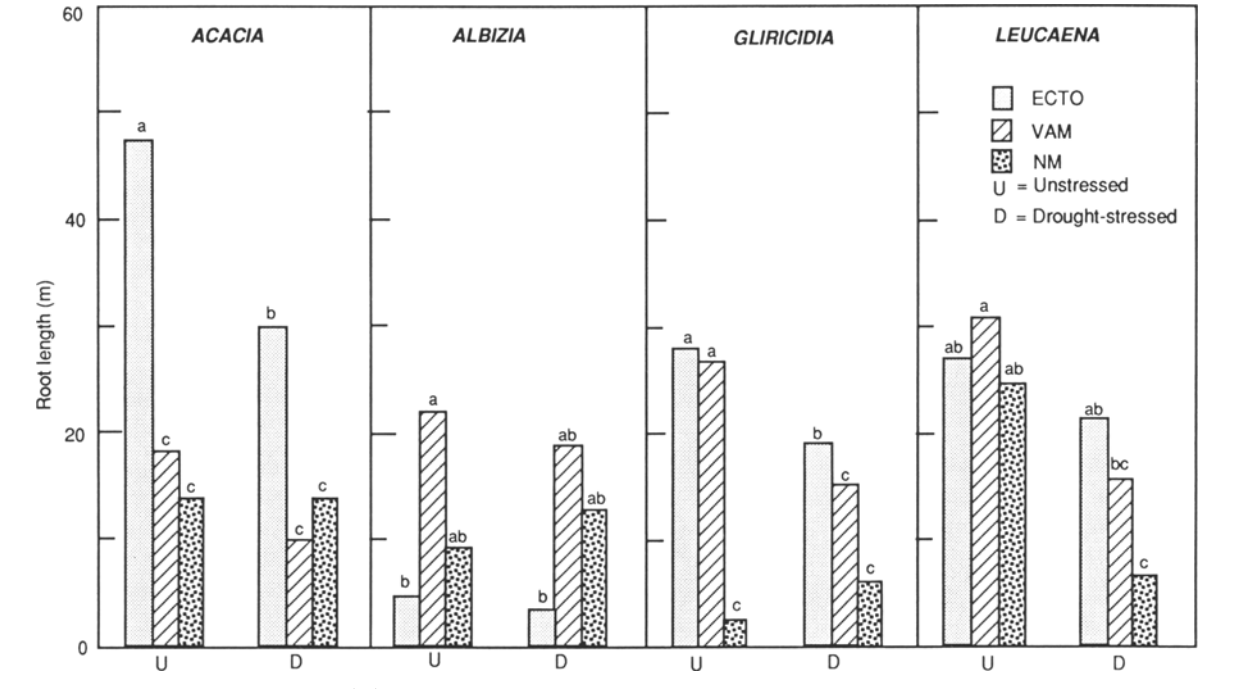

Fig. 3. Root length of unstressed (U) and drought-stressed (D) ectomycorrhizal (ECTO), vesicular-arbuscular mycorrhizal (VAM) and non-mycorrhizal (NM) of four leguminous woody seedlings at the end of experiment. *Bars* without the same letter above are significantly different at $p < 0.05$ according to Duncan's multiple range test.

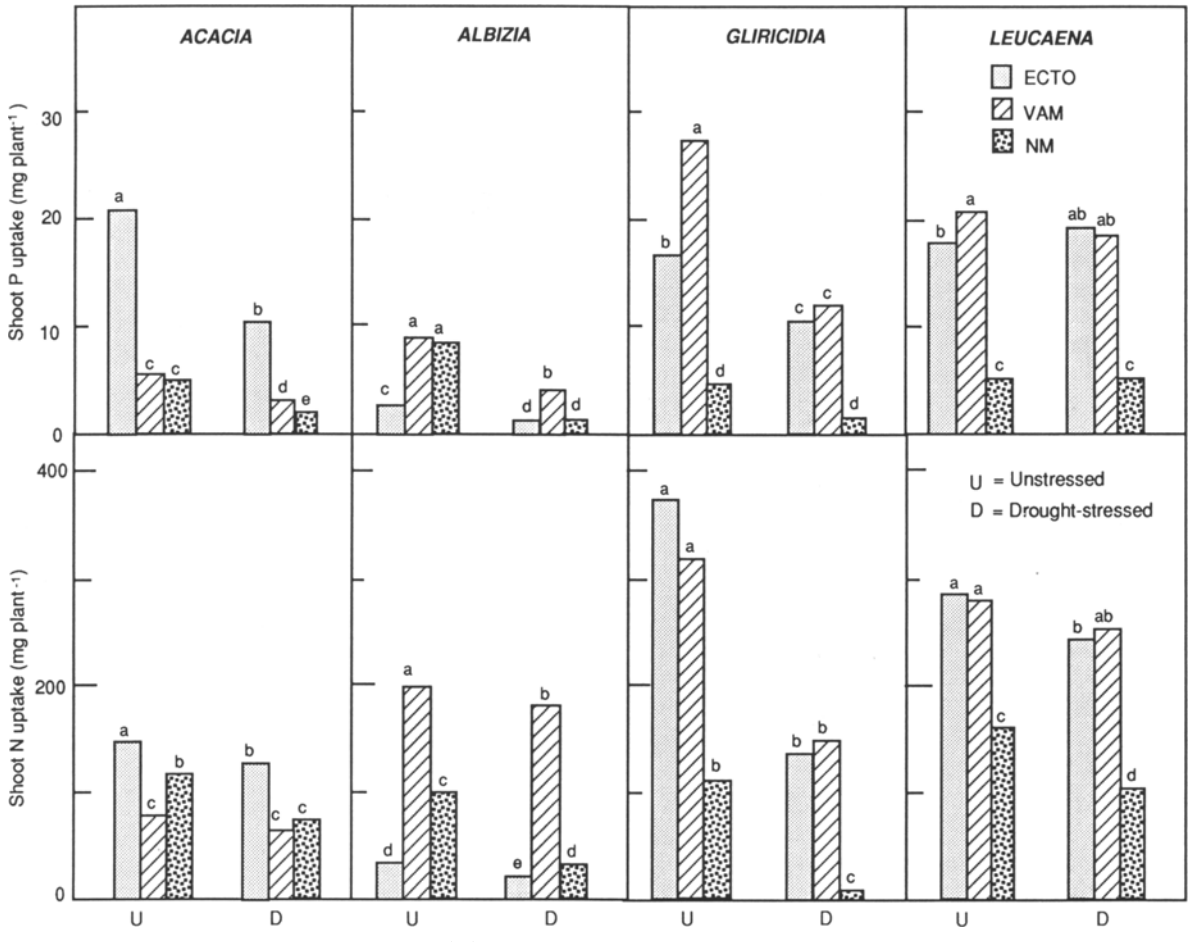

Fig. 4. Shoot P and N uptake of unstressed (U) and drought-stressed (D) ectomycorrhizal (ECTO), vesicular-arbuscular mycorrhizal (VAM) and non-mycorrhizal (NM) leguminous woody seedlings at the end of experiment. *Bars* without the same letter above are significantly different at $p < 0.05$ according to Duncan's multiple range test.

growth characteristics and nutrient uptake of different mycorrhizas under either watering regime were not significantly different, the data for both mycorrhizas were combined for further comparisons. Linear regression analysis (Table 2) revealed that growth depends on nutrients under limiting conditions and mycorrhizal effects on N uptake are almost mediated via P nutrition.

Water relations

Under unstressed conditions, soil moisture content and xylem pressure potential were generally unaffected by either mycorrhizal inoculation (Table 3). Both types of mycorrhizal inoculation results in higher RWC of unstressed plants of *Acacia* and *Gliricidia* than their NM plants. In

Table 2. Correlation coefficients between growth characteristics, P uptake and nitrogen uptake of all the leguminous plants combined

	Shoot dry weight	Root dry weight	Root length	P
P	0.90***	0.85***	0.72***	–
N	0.94***	9.92***	0.60***	0.83***

Values significantly correlated *** $p < 0.001$.

Table 3. Soil moisture content, xylem pressure potential and leaf relative water content (RWC) of unstressed and drought-stressed ectomycorrhizal (ECTO), vesicular-arbuscular mycorrhizal (VAM) and non-mycorrhizal (NM) leguminous woody seedlings at the end of experiment

	Soil moisture content (%)	Xylem pressure potential (MPa)	Leaf RWC (%)
Acacia auriculiformis			
Unstressed			
ECTO	20.11a	−1.38ab	94.28a
VAM	21.57a	−1.65b	92.38a
NM	20.32a	−1.15a	87.70b
Drought-stressed			
ECTO	9.68c	−2.52c	78.35c
VAM	9.68c	−2.50c	65.83e
NM	10.40b	−1.30ab	75.14d
Albizia lebbeck			
Unstressed			
ECTO	20.63a	−1.23a	98.80ab
VAM	21.32a	−1.23a	99.36a
NM	20.90a	−1.17a	98.47bc
Drought-stressed			
ECTO	12.97b	−1.19a	98.44bc
VAM	11.97b	−1.87c	98.83ab
NM	12.69b	−1.57b	98.47bc
Gliricidia sepium			
Unstressed			
ECTO	20.11a	−0.90a	82.72b
VAM	20.64a	−1.33ab	88.66a
NM	21.53a	−0.92a	81.17c
Drought-stressed			
ECTO	10.64b	−1.53b	77.75d
VAM	7.64c	−1.47b	77.62d
NM	7.86c	−1.50b	74.87e
Leucaena leucocephala			
Unstressed			
ECTO	19.90a	−1.82ab	83.80a
VAM	20.80a	−1.96ab	84.87a
NM	21.07a	−1.68a	83.16a
Drought-stressed			
ECTO	11.17b	−2.3bc	54.76d
VAM	8.02c	−2.73c	75.35b
NM	8.64c	−2.55c	65.48c

Means for each leguminous species within the same vertical column followed by different letter are significantly different at $p < 0.05$ according to Duncan's multiple range test.

either *Albizia* or *Leucaena*, the RWC for mycorrhizal and NM plants were similar.

Overall, drought stress decreased the RWC of all plants except for *Albizia* which had comparable values under both watering regimes. Compared to the unstressed plants, drought stress reduced the xylem pressure potential of all *Leucaena* and mycorrhizal *Acacia* plants. Although drought stress reduced the xylem pressure potential of *Albizia*, it was minimal com-

pared to those of *Acacia* and *Leucaena*. In *Gliricidia*, xylem pressure potential was not really affected by drought stress.

Mycorrhizal dependency (MD) and drought response index (DRI)

Under both types of mycorrhizal inoculations and watering regimes, *Gliricidia* was the most strongly mycorrhizal dependent (Fig. 5) but with the lowest DRI (Table 4). *Leucaena* had similar or comparable MD and DRI with *Acacia* and *Albizia* respectively under ectomycorrhizal and VAM inoculations. The lowest MD in either *Acacia* or *Albizia* may be attributed to their lowest infection by respective mycorrhizas. DRI was not affected by mycorrhizal inoculation in *Gliricidia* and *Leucaena* (Table 4). It tended to improve DRI of *Acacia* and *Albizia* (VAM) but the effect was not significant. Although there was considerable variation in DRI among the plants, it was inversely related to MD (Fig. 6).

Discussion

In this study, analysis of nutrient uptake reaffirms that growth stimulation in both unstressed and drought-stressed mycorrhizal plants compared to their NM plants may be attributed to improved accumulation of nutrients, particularly P (Graham and Syvertsen, 1985; Huang et al., 1985; Mejstrik, 1975; Osonubi, 1989; Osonubi et al., 1990; Rhodes and Gerdemann, 1980; Smith and Gianinazzi-Pearson, 1988). Shoot and root dry weights of *Acacia* and *Albizia* were depressed by VAM and ectomycorrhizal inoculation respectively. In *Acacia*, growth depression by VAM may be attributed to carbon drain from the host to the fungus (Bethlenfalvay

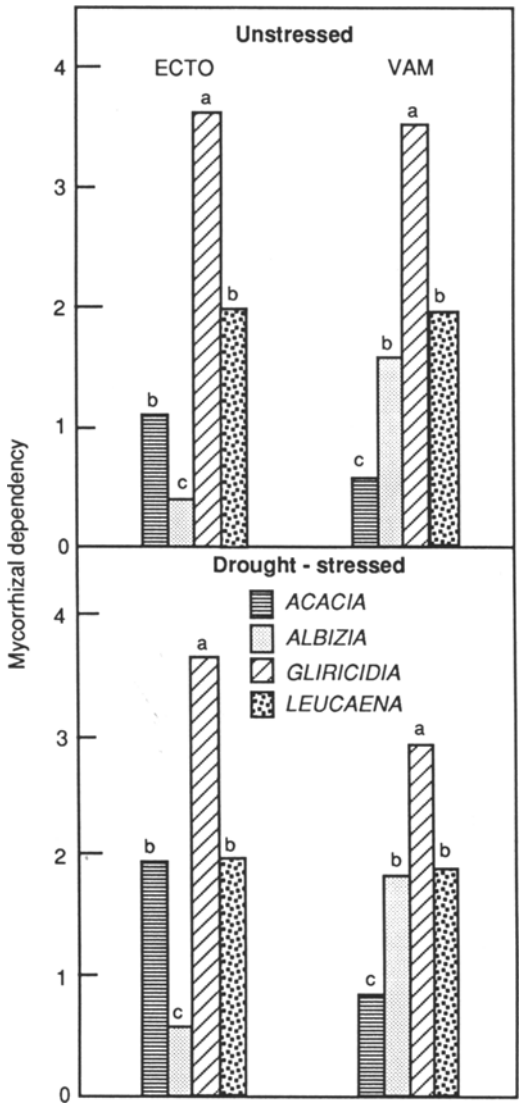

Fig. 5. Mycorrhizal dependency of unstressed (U) and drought-stressed (D) ectomycorrhizal (ECTO) and vesicular-arbuscular mycorrhizal (VAM) of four woody seedlings at the end of experiment. *Bars* without the same letter above are significantly different at $p < 0.05$ according to Duncan's multiple range test.

Table 4. Effect of mycorrhizal on drought response index (DRI) of four leguminous woody plants

Treatment	*Acacia auriculiformis*	*Albizia lebbeck*	*Gliricidia sepium*	*Leucaena leucocephala*
ECTO	0.69	0.47b	0.47	0.68
VAM	0.75	0.64a	0.45	0.66
NM	0.57	0.56ab	0.42	0.72

Means on the same vertical column followed by different letters are significantly different at $p < 0.05$ according to Duncan's multiple range test. Lack of letters indicates no significant difference. ECTO: Ectomycorrhizal, VAM: vesicular-arbuscular mycorrhizal, NM: non-mycorrhizal.

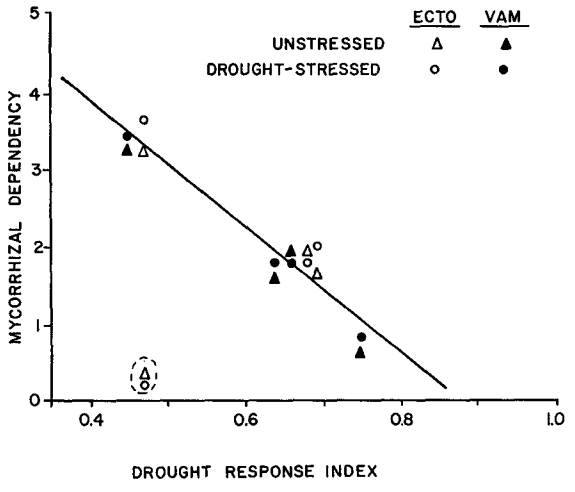

Fig. 6. Relationship between mycorrhizal dependency (MD) and drought response index (DRI). Data from Table 4 and Fig. 5. ECTO: ectomycorrhizal, VAM: vesicular-arbuscular mycorrhizal. The data points enclosed by dashed lines represent values for ectomycorrhizal *Albizia* and were not included for regression analysis. MD = 7.16–8.16 DRI, r = −0.96.

et al., 1982; Buwalda and Goh, 1982; Ho and Trappe, 1973) as a cost for greater P uptake than NM plants (Kucey and Paul, 1982; Snellgrove et al., 1982), particularly under drought-stressed conditions (Figs. 2 and 4). In *Albizia*, the reduced growth (Figs. 1 and 2) does not directly suggest carbon drain mechanism but indirectly suggests facultative parasitic nature of the ectomycorrhiza. Our suggestion is reinforced by the fact that the ectomycorrhizal inoculation never resulted in comparable but reduced P and N uptake compared with NM plants except for the P uptake in drought-stressed plants (Fig. 4 cf. VAM *Acacia*). Also visual observations of root morphology showed poorer growth in ectomycorrhizal plants compared to NM plants.

In spite of growth stimulation by ectomycorrhiza, mantle sheath on lateral roots was absent but with Hartig net formation separating cortical cells. The reason for this is unclear. Although, similar observations on the absence or scarcity of visible ectomycorrhizal infection early in the association have been attributed to alteration in root system physiology by the fungus (Daughtridge et al., 1986; Reid et al., 1983). In the present study, the absence of mantle sheath may be due to the fact that this ectomycorrhiza is temperate in origin and has an optimum temperature for mantle growth which is far below the experimental temperature, which at times may be as high as 50°C, in the polyethylene bags (Redhead, 1982). Alternatively, the strain of the ectomycorrhiza used in the present study may not be the mantle sheath forming type but can develop Hartig net in the roots as reported for *Pinus banksiana* inoculated with variant strains of *Laccaria bicolor* (Wong et al., 1989).

Root growth is stimulated by ectomycorrhizal infection (Kottke and Oberwinkler, 1986) or VAM infection (Graham and Syvertsen, 1985). In the present study, root growth in both unstressed and drought-stressed plants corresponded with the nutrient uptake (Table 2, and Figs. 3 and 4) except *Leucaena*. In this plant (*Leucaena*), root growth in all the treatments was not significantly different from each other except for drought-stressed NM plants. One would have expected comparable P uptake in all the treatments with comparable root growth. However, the P uptake of unstressed NM plants was considerably less and only comparable to drought-stressed NM plants. Although there is no direct evidence of hyphal uptake of water and nutrients in this study, the results with *Leucaena* indirectly suggest the involvement of fungal hyphae in water transport to roots (Allen, 1982; Read and Boyd, 1982) which is likely to have enhanced or overcome the slow diffusion of P in the drying soil (Nelsen and Safir, 1982).

Drought stress reduced the xylem pressure potential of mycorrhizal *Acacia* more than its NM plants (Table 3). Since dry biomass accumulation in drought-stressed ectomycorrhizal plants is higher than that in drought-stressed NM plants (Fig. 2), it can be suggested that the more negative values of xylem pressure potential in mycorrhizal plants may be attributed to maintenance of water potential gradients for water uptake in drying soil (Osonubi, 1989; and Osonubi and others, unpublished data). This is likely to be the case as the xylem pressure potential and RWC values were obtained without any visible signs of wilting. It then follows that mycorrhizal *Acacia* can tolerate lower xylem pressure potential and larger water losses with-

out wilting. This may provide the explanation for the reduced xylem pressure potential and RWC in drought-stressed *Leucaena* plants compared to the unstressed plants. Osmotic adjustment for turgor maintenance in these mycorrhizal plants may be another possible explanation (Allen and Boosalis, 1983; Osonubi, 1989). The occurrence of comparable xylem pressure potential between drought-stressed mycorrhizal, NM and unstressed plants of Gliricidia is likely to be in response to a strategy for regulating RWC to prevent the deleterious effects of water stress. Specifically, movements of adaxial surfaces of individual leaflets of unstressed and drought-stressed *Albizia*, *Gliricidia* and *Leucaena* have been visually observed towards one another between 1200 and 1600 h, which incidentally is the period of maximum stress. These leaf or leaflet movements have been described as strategies of avoiding incident light which may cause severe water stress effects on plants (Huang et al., 1985; Prichard and Forseth, 1988).

Drought response index (DRI) has not been used previously to assess MD. Differences in MD of plant species have been based on ability of NM plants to take up P from low P soils (Hall, 1975; Menge et al., 1978). Others have suggested that species with fewer lateral roots and shorter root systems are more dependent on mycorrhiza than those with greater lateral roots and finer root systems (Pope et al., 1983). More recently, the ability of roots to take up water (hydraulic conductivity) among root stocks has been added to the determinants of MD (Graham and Syvertsen, 1985). In this study, DRI of mycorrhizal plants provides another insight into the nature of mycorrhizal dependency in a low P soil as it relates inversely to MD (Fig. 6). The higher the MD, the lower the DRI. The only exception to this determinant is in mycorrhizal plants with the mycorrhiza acting as a facultative parasite, e.g. ectomycorrhizal *Albizia*. Although mycorrhizal inoculation does not seem to improve the DRI of plants, it however enhanced their MD and growth through the nutrient uptake under stressed and unstressed conditions.

All the four leguminous woody seedlings benefited from symbiotic associations with either ecto- or VA mycorrhiza or both. *Albizia*, *Gliricidia* and *Leucaena* are currently used for alley farming. If these responses with respect to nutrient uptake and growth are reproducible in the field conditions, efficient mycorrhizal management of these species may form an integral component of the closed, nutrient cycle of alley farming.

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References

Allen E B and Allen M F 1986 Water relations of xeric grasses in the field: Interactions of mycorrhizal and competition. New Phytol. 104, 559–571.

Allen M F 1982 Influence of vesicular-arbuscular mycorrhizae on water movement through *Bouteloua gracilis* (H.B.K.) Lag ex Steud. New Phytol. 91, 191–196.

Allen M F and Boosalis M G 1983 Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. New Phytol. 93, 67–76.

Bethlenfalvay G J, Brown M S and Pacovsky R S 1982 Parasitic and mutualistic associations between a mycorrhizal fungus and soybean: Development of the host plant. Phytopathology 72, 889–893.

Brundrett M C, Piché Y and Peterson R L 1984 A new method for observing the morphology of vesicular-arbuscular mycorrhizae. Can. J. Bot. 62, 2128–2134.

Buwalda J G and Goh K M 1982 Host-fungus competition for carbon as a cause of growth depressions in vesicular-arbuscular mycorrhizal ryegrass. Soil Biol. Biochem. 14, 103–106.

Crush J R 1976 Endomycorrhizas and legume growth in some soils of the MacKenzie Basin, Canterbury, New Zealand. N.Z.J. Agric. Res. 19, 473–476.

Daughtridge A T, Pallardy S G, Garret H G and Sander I L 1986 Growth analysis of mycorrhizal and non-mycorrhizal black oak (*Quercus velutina* Lam.) seedlings. New Phytol. 103, 473–480.

Ekwebelam S A and Reid C P P 1983 Effect of light, nitrogen fertilization, and mycorrhizal fungi on growth and photosynthesis of lodgepole pine seedlings. Can. J. For. Res. 11, 1099–1106.

Gibson F, Frances M F and Deacon J W 1988 Effects of

microwave treatment of soil on growth of birch (*Betula pendula*) seedlings and infection of them by ectomycorrhizal fungi. New Phytol. 108, 189–204.

Graham J H and Syvertsen J P 1984 Influence of vesicular-arbuscular mycorrhiza on the hydraulic conductivity of roots of two citrus rootstocks. New Phytol. 97, 277–284.

Graham J H and Syvertsen J P 1985 Host determinants of mycorrhizal dependency of citrus rootstock seedlings. New Phytol. 101, 667–676.

Hall I R 1975 Endomycorrhizas of *Metrosideros umbellata* and *Weinmannia racemosa*. N.Z.J. Bot. 13, 463–472.

Hardie K and Leyton L 1981 The influence of vesicular-arbuscular mycorrhiza on growth and water relations of red clover. I. In phosphate deficient soil. New Phytol. 89, 599–608.

Harley J L and Smith S E 1983 Mycorrhizal Symbiosis. London, Academic Press.

Hayman D S 1986 Mycorrhizae of nitrogen-fixing legumes. MIRCEN J. Appl. Microbiol. Biotech. 2, 121–145.

Henderson J C and Davies F T Jr 1990 Drought acclimation and the morphology of mycorrhizal *Rosa hybrida* L. cv. 'Ferdy' is independent of leaf elemental content. New Phytol. 115, 503–510.

Hewlett J D and Kramer J P 1963 The measurement of water deficits in broad leaf plants. Protoplasma 57, 381–391.

Ho I and Trappe J M 1973 Translocation of ^{14}C from *Festuca* plants to endomycorrhizal fungi. Nature-New Biol. 244, 30–31.

Huang R S, Smith W K and Yost R S 1985 Influence of vesicular-arbuscular mycorrhiza on growth, water relations, and leaf orientation in *Leucaena leucocephala* (Lam.) de Wit. New Phytol. 99, 229–243.

International Institute of Tropical Agriculture (IITA) 1982 Selected Methods for Soil and Plant Analysis. Manual Series No. 7, IITA, Nigeria.

Kang B T and Wilson G F 1987 The development of alley farming as a promising agroforestry technology. *In* Agroforestry: A Decade of Development. Eds. H A Steppler and P K R Nair. pp 227–243. ICRAF, Nairobi, Kenya.

Koide R 1985 The nature of growth depressions in sunflower caused by vesicular-arbuscular mycorrhizal infection. New Phytol. 99, 449–462.

Kottke I and Oberwinkler F 1986 Mycorrhiza of forest trees: Structure and function. Trees 1, 1–24.

Kozlowski TT 1982 Water supply and tree growth. Part I. Water deficits. For. Abstr. 43, 57–95.

Kramer P J and Kozlowski T T 1979 Physiology of Woody Plants. Academic Press, New York, London.

Kucey R M N and Paul E A 1982 Carbon flow, photosynthesis and N_2 fixation in mycorrhizal and nodulated fababeans (*Vicia faba* L.). Soil Biol. Biochem. 14, 407–412.

Levy Y and Krikun J 1980 Effect of vesicular-arbuscular mycorrhiza on *Citrus jambhiri* water relations. New Phytol. 85, 25–36.

Mcgonigle T P, Miller M H, Evans D G, Fairchild G L and Swan J A 1990 A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115, 495–502.

Marschner H 1986 Mineral Nutrition of Higher Plants. Academic Press, London.

Mejstrik V K 1975 The effect of mycorrhizal infection of *Pinus sylvestris* and *Picea abies* by two *Boletus* species on the accumulation of phosphorus. New Phytol. 74, 455–459.

Menge J A, Johnson E L V and Platt R G 1978 Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. New Phytol. 81, 553–559.

Michelsen A and Rosendahl S 1990 The effect of VA mycorrhizal fungi, phosphorus and drought stress on the growth of *Acacia nilotica* and *Leucaena leucocephala* seedlings. Plant and Soil 124, 7–13.

Mosse B 1973 Plant growth responses to vesicular-arbuscular mycorrhiza. IV. In soils given additional phosphate. New Phytol. 72, 127–136.

Mulongoy K, Callens A and Okogun J A 1988 Differences in mycorrhizal infection and P uptake of sweet potato cultivars (*Ipomea batata* L.) during their early growth in three soils. Biol. Fertil. Soils 7, 7–10.

Nelsen C E and Safir G R 1982 Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Planta 154, 407–413.

Osonubi O 1989 Osmotic adjustment in mycorrhizal *Gmelina arborea* Roxb. seedlings. Func. Ecol. 3, 143–151.

Osonubi O, Okon I E and Bamiduro T A 1990 Effect of different fungal inoculation periods on performance of *Gmelina* seedlings under dry soil conditions. For. Ecol. Manage. 37, 223–232.

Pachlewski R 1967 Investigations on pure culture of mycorrhizal fungi of pine (*Pinus sylvestris* L.). Forest Research Institute, Warsaw, 193 p.

Parke J L, Linderman R G and Black C H 1983 The role of ectomycorrhizas in drought tolerance of Douglas-fir seedlings. New Phytol. 95, 83–95.

Pope P E, Chaney W R, Rhodes J D and Weedhead S H 1983 The mycorrhizal dependency of four hardwood tree species. Can. J. Bot. 61, 412–417.

Prichard J M and Forseth I N 1988 Rapid leaf movement, microclimate, and water relations of two temperate legumes in three contrasting habitats. Am. J. Bot. 75, 1201–1211.

Read D J and Boyd R C 1986 Water relations of mycorrhizal fungi and their host plants. *In* Water, Fungi and Plants. Eds. P G Ayres and L Boddy. pp 287–303. Cambridge University Press, Cambridge.

Redhead J F 1982 Ectomycorrhizae in the tropics. *In* Microbiology of Tropical Soils and Plant Productivity. Eds. Y R Dommergues and H G Diem. pp. 253–269. Nijhoff/Junk Publishers, The Hague, The Netherlands.

Reid C P P, Kidd F A and Ekwebelam S A 1983 Nitrogen nutrition, photosynthesis and carbon allocation in ectomycorrhizal pine. Plant and Soil 71, 415–432.

Rhodes L H and Gerdemann J W 1980 Nutrient translocation in vesicular-arbuscular mycorrhizae. *In* Cellular Interactions in Symbiosis and Parasitism. Eds. C B Cook, P W Pappas and E D Rudolph. pp 173–195. Ohio State University Press, Columbus.

Smith S E and Gianinazzi-Pearson V 1988 Physiological interactions between symbionts in vesicular-arbuscular

mycorrhizal plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39, 221–244.

Snellgrove R C, Splittstosser W E, Stribley D P and Tinker P B 1982 The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. New Phytol. 92, 75–87.

Tennant D 1975 A test of modified line intersect method of estimating root length. J. Ecol. 63, 995–1001.

Wong K K Y, Piché Y, Montpetit D and Kropp B R 1989 Differences in the colonization of *Pinus banksiana* roots by sib-monokaryotic and dikaryotic strains of ectomycorrhizal *Laccaria bicolor*. Can. J. Bot. 67, 1717–1726.