WATER RELATIONS AND GAS EXCHANGE OF MYCORRHIZAL *LEUCAENA LEUCOCEPHALA* SEEDLINGS

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DIXON, R.K., RAO, M.V. & GARG, V.K. 1994. Water relations and gas exchange of mychorrhizal *Leucaena leucocephala* seedlings. *Leucaena leucocephala* seedlings were inoculated with four species of vesicular-arbuscular mycorrhizal (VAM) fungi, *Gigaspora margarita, Glomus deserticola, Glomus etunicatum* and *Glomus intraradices*, and two species of ectomycorrhizal fungi, *Pisolithus tinctorius* and *Laccaria laccata*. After 16 weeks in a glasshouse, plants inoculated with VAM fungi were significantly larger (biomass and leaf area) than non-inoculated control seedlings. Adequate VAM colonization was observed on root systems of plants inoculated with *Gigaspora* and *Glomus* species. Plants inoculated with ectomycorrhizal fungi were non-mycorrhizal but were larger in biomass than non-inoculated control seedlings. Phosphorus concentration of mycorrhizal seedlings was significantly greater than non-mycorrhizal plants. Leaf water potential, leaf stomatal conductance and photosynthesis of seedlings were measured at mid-light during pre-, mid-, and post-water stress treatments. Although larger in biomass and leaf area, the VAM seedlings maintained slightly greater leaf water potential, leaf stomatal conductance and photosynthesis relative to the non-mycorrhizal plants at the peak of the drought. After re-watering the growth medium, leaf water potential, leaf conductance, and photosynthesis of the VAM seedlings were significantly greater compared to non-mycorrhizal plants. These data suggest that VAM fungi help *Leucaena leucocephala* to avoid drought stress.

Key words: *Glomus - Gigaspora - Pisolithus - Laccaria* - water stress

DIXON, R.K., RAO, M.V. & GARG, V.K. 1994. Kaitan air dan pertukaran gas anak benih *Leucaena leucocephala* yang dijangkiti mikoriza. Anak benih *Leucaena leucocephala* diinokulasi dengan empat spesies kulat mikoriza vesikular-arbuskular (VAM), iaitu *Gigaspora margarita, Glomus deserticola, Glomus etunicatum* dan *Glomus intraradices* dan dua spesies kulat ektomikoriza iaitu *Pisolithus tinctorius* dan *Laccaria laccata*. Setelah berada di dalam rumah kaca selama 16 minggu, biojisim dan keluasan daun tumbuhan yang diinokulasi dengan kulat VAM nyata sekali lebih

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besar daripada anak benih kawalan yang tidak diinokulasi. Pengkolonian VAM yang secukupnya kelihatan pada sistem akar tumbuhan yang diinokulasikan dengan spesies *Gigaspora* dan *Glomus*. Tumbuhan yang diinokulasikan dengan kulat ektomikoriza tidak dijangkiti mikoriza tetapi mempunyai biojisim yang lebih besar daripada anak benih kawalan yang tidak diinokulasikan. Kepekatan fosforus anak benih yang dijangkiti mikoriza nyata sekali lebih tinggi daripada tumbuhan yang tidak dijangkiti mikoriza. Keupayaan air daun, konduktans stomata daun dan fotosintesis anak benih diukur pada waktu tengah hari sebelum rawatan tegasan, semasa pertengahan rawatan tegasan dan selepas rawatan tegasan air. Pada puncak kemarau, anak benih yang dijangkiti VAM mengekalkan keupayaan air daun, konduktans stomata daun dan fotosintesis yang lebih tinggi sedikit berbanding dengan tumbuhan yang tidak dijangkiti mikoriza. Setelah bahantara pertumbuhan dibubuh air semula, keupayaan air daun, konduktans daun dan fotosintesis anak benih yang dijangkiti VAM nyata sekali lebih tinggi berbanding dengan tumbuhan yang tidak dijangkiti VAM. Data ini menunjukkan bahawa kulat VAM membantu *Leucaena leucocephala* mengelak daripada tegasan air.

Introduction

Over 2 billion *ha* of degraded lands and substandard soils occur worldwide (Grainger 1988, Jain *et al.* 1989). Approximately 30% of the world's land area are deserts (Hellden 1992). Reclamation and revegetation of degraded lands are a global priority (Winjum *et al.* 1992). Excessive seedling mortality and poor juvenile growth are attributable to edaphic and climatic factors, especially water stress over a range of site conditions (Schulze 1986, Hellden 1992).

Inoculation of tree seedlings with mycorrhizal fungi, both vesicular-arbuscular mycorrhizae (VAM) and ectomycorrhizae, significantly improve survival and juvenile growth (Hayman 1983, Marx *et al.* 1991). Preliminary assessments also reveal that inoculation of tropical trees, particularly fast-growing nitrogen-fixing species, with VAM fungi will improve survival and juvenile growth (Osonubi *et al.* 1991). The response of tree seedlings to VAM fungi has been attributed to improved phosphorus (P) and microelement nutrition (Harley & Smith 1983, Habte & Fox 1992), changes in shoot-root growth regulator relations (Dixon *et al.*1988), and possibly improved plant water relations (Huang *et al.* 1985). Species and genotypes of mycorrhizae yield differential benefits to the host (Rao *et al.* 1989, Lamhamedi *et al.* 1992) but this relationship is poorly understood.

The role of ectomycorrhizal symbiosis in tree-water relations and gas exchange has been partially elucidated (Parke *et al.* 1983, Brownlee *et al.* 1985, Lamhamedi *et al.* 1992). Water stress is reduced in *Eucalyptus, Pinus, Pseudotsuga* and *Quercus* seedlings colonized with selected ectomycorrhizal symbionts (Dixon *et al.* 1983, Parke *et al.* 1983, Dixon & Hiol Hiol 1992). Improved tree-water relations associated with mycorrhizal root systems may result from: 1) an enlarged root-fungus absorbing area (Dixon *et al.* 1983), 2) reduced root shrinkage (Reid 1979), 3) functioning of the fungus as low resistance path way for water movement to the root cortex (Duddridge *et al.*1980), and, 4) stimulation of root growth (Dixon *et al.* 1980). Photosynthesis and carbon accretion of seedlings inoculated with ectomycorrhizal fungi is generally greater than in plants without mycorrhizae (Ekwebelam & Reid 1983, Dosskey *et al.* 1990).

Little information is available about the role of VAM in the water relations of fast-growing nitrogen-fixing tree species (Michelsen & Rosendahl 1990, Osonubi *et al.* 1991). Drought which leads to wilting of leaf tissue reduces biomass production in *Acacia*, *Leucaena* and *Prosopis* (Felker *et al.* 1983, Michelsen & Rosendahl 1990). Assessments in irrigated, fertile soil suggest that the water relations of *Acacia, Leucaena* and *Prosopis* species are altered following inoculation with selected VAM (Huang *et al.* 1985, Osonubi *et al.* 1991). *Acacia* seedlings exhibit drought tolerance characteristics such as osmotic adjustment (Michelsen & Rosendahl 1990), whereas *Leucaena* species appear to avoid drought (Samson & Pacardo 1983, Huang *et al.* 1985). Temporal patterns of *Leucaena leucocephala* water relations and gas exchange have been surveyed under irrigated conditions (Natarahn *et al.* 1985) but not under conditions of a cyclic drought.

The objectives of this study were to: 1) evaluate root and shoot morphology (biomass, leaf area, mycorrhizal associations) of *Leucaena leucocephala* Lam. de Wit inoculated with four VAM and two ectomycorrhizal fungi, and, 2) compare their leaf water potential, leaf conductance and photosynthesis over the course of a short-term cyclic drought and subsequent recovery period.

Materials and methods

Seedling production

Leucaena leucocephala seedlings were propagated in 2-*l* containers, and cultured in a greenhouse using methods described by Dixon and Hiol Hiol (1992). Seeds of *Leucaena leucocephala*, variety K-8, were obtained from the Nitrogen-Fixing Tree Association, Waimanalo, Hawaii, USA. Following scarification with concentrated H_2SO_4, seeds were soaked in sterile-deionized water for 24 *h*, treated with a suspension of *Rhizobium* sp. (strain TAL 1145), and transferred to containers containing the growth medium. Uniform seedling germination was complete in seven days.

The growth medium was a sandy loam with a pH of 4.8, and P, Ca, K, Mg, Zn, B and Mo contents of 6, 9,13, 3, 0.3, 0.4 and 0.01 *ppm* respectively. Organic matter content of the soil was 1.5%. All plants received a balanced nutrient solution weekly prior to the short-term drought (Hoagland & Arnon 1950). Glasshouse ambient environment during the seedling production phase included a 35°*C* average mid-day temperature, 85% average mid-day relative humidity (RH), and a 14-*h* photoperiod. During the short-term drought glasshouse ambient temperature, relative humidity and photosynthetically active radiation (PAR) ranged from 26 - 37°*C*, 37 - 82%, and 510 - 1590 μ *mol* m^{-2} s^{-1} respectively (Dixon & Hiol Hiol 1992). The glasshouse environment and cultural conditions were based on earlier studies of *Leucaena* culture (Rao *et al.* 1989).

Cultures of mycorrhizae and inoculation

Four VAM fungi were employed in this study: *Gigaspora margarita* Becker and Hall, *Glomus etunicatum* Becker and Gerdemann, *Glomus deserticola* Trappe, Bloss & Menge and *Glomus intraradices* Schenck and Smith. The *Gigaspora* and *Glomus* species were collected from soils of mixed stands of *Acacia* and *Prosopis* along Delhi Ridge, Delhi, India (Kaushik *et al.* 1992). Prior to preparation of inoculum for this study the fungal cultures were maintained in microplots with either *Glycine* or *Sorghum* sp. as hosts (Dixon 1988). Inoculum for this study was produced in pot culture with sorghum (*Sorghum bicolor*) as the plant host (Menge *et al.* 1978). After 16 weeks spores or root particles (*e.g.*, *G. intraradices*) were sieved and extracted from the soil mix using techniques described by Gerdemann and Nicholson (1963). Approximately 200 VAM propagules were placed in the growth medium 2- 5 *cm* below the seed at the time of planting.

The isolates of *Pisolithus tinctorius* (Pers.) Coker & Couch and *Laccaria laccata* (Scop.:Fr.) B & Br. were collected from pine (*Pinus*) stands in Athens, GA and Gainesville, FL, USA respectively. Vegetative mycelial inoculum of *P. tinctorius* and *L. laccata* were grown in a liquid substrate of modified Melin-Norkrans solution using techniques described by Marx (1969). Vegetative inoculum was leached with deionized water, macerated, and 0.5 *g* (fresh weight) was mixed into the seedling growth medium (1:30, v:v) at the time of planting. Seedlings with and without VAM and ectomycorrhizal fungi were established in the experiment. The non-inoculated *L. leucocephala* seedlings, maintained as controls, received inoculum leachate to standardize microflora of the rhizosphere (Marx 1969). Otherwise, the non-inoculated control seedlings received the same cultural treatments as the inoculated plants.

Soil water treatments

Seedling growth medium was watered daily to field capacity for 14 weeks following planlet emergence. Relatively uniform soil water potential was achieved by weighing pots and re-watering to the same weight (Dixon & Hiol Hiol 1992). Soil water potential was also monitored using ceramic cup psychrometers (Bildusas *et al.* 1985). After 14 weeks the seedlings were subjected to water stress. Water was withheld from seedlings until soil water potential reached - 1.5 *MPa* (peak-stress on day 9). Plant growth medium was then re-watered to field capacity.

Seedling water relations and gas exchange measurements

During the short-term drought seedling leaf water potential (Ψ), photosynthesis rate (Ps), and leaf stomatal conductance (K) were measured twice daily on day 1 (pre-stress) and day 9 (peak-stress) of the short-term drought. Five seedlings were randomly selected in each treatment plot (replication) for the

water relations measurements. The phyllotaxy, developmental stage and leaf orientation of the *L. leucocephala* seedlings were the same in each treatment. Thus, age or condition of leaflet tissue were not a source of variation in this experiment (Huang *et al.* 1985). Leaf water potential (Ψ) was measured from detached petioles using a pressure chamber (PMS Instrument Company, Corvallis, OR, USA) at 6:00 and 13:00 using methods described by Dixon *et al.* (1983). On the same day at 10: 00 and 14:00, photosynthesis rate (Ps) and leaf stomatal conductance (K) were measured on leaflets of seedlings in the same treatments using the ADC LCA.2 portable infrared carbon dioxide analyzer (The Analytical Development Co., Ltd., Hoddesdon, Herts, England, UK), using methods and formulas described by Combs *et al.* (1985). Seedlings were re-watered and a full set of water relations measurements were completed on day 10 to evaluate seedling recovery from water stress.

Seedling morphological measurements

Seedlings were harvested and analyzed after the short-term drought. Measurements included seedling dry weight and leaf surface area. Seedling dry weight was obtained following oven drying at 75°C for 72 *h*. Leaf surface area was measured using a portable area meter (L1-Cor Model L1-3000, Lincoln, NE, USA). Phosphorus concentration in plant leaf tissue was determined by the molybdate blue method (Murphy & Riley 1962). A sub-sample of five seedlings was collected in each treatment plot (replication) and 10% of the primary lateral roots were randomly excised from each plant. Lateral roots were cleared and stained (Phillips & Hayman 1970) and evaluated to determine VAM colonization using methods described by Dixon (1988). Ectomycorrhizal colonization was evaluated using methods described by Lamhamedi *et al.* (1992). The presence of Hartig net and fungal mantle was determined microscopically after excising ectomycorrhizal short roots from seedlings (Marx 1969).

Experimental design and statistical analysis

The experiment was implemented and analyzed as a split-plot design (Steel & Torrie 1980). Vesicular-arbuscular mycorrhizal (4), ectomycorrhizal (2) and non-inoculated (2) seedling treatment whole plots were replicated 15 times each. The three sampling dates (pre-stress, peak-stress and post-stress) were the sub-plots. Data were subjected to analysis of variance and the least significant difference test ($p = 0.05$).

Results

Inoculation of the *Leucaena leucocephala* seedlings with *Gigaspora margarita* and the three *Glomus* species resulted in abundant VAM development but the degree of colonization varied (Table 1). Plants inoculated with *Glomus intraradices* had the most extensive VAM development. True ectomycorrhiza was not observed on

seedling root systems inoculated with *P. tinctorius* or *L. laccata*, but a loose fungal mantle of hyphae surrounded the feeder roots. A Hartig net was not observed in seedling short roots.

Leucaena leucocephala seedlings inoculated with *Gigaspora margarita, Glomus deserticola* and *G.etunicatum* were significantly larger in total dry weight, relative to non-mycorrhizal plants (Table 1). Seedlings inoculated with *P. tinctorius* and *L. laccata* were significantly larger than the non-inoculated control plants but smaller in size than those inoculated with VAM fungi. Inoculation of *L. leucocephala* with VAM and ectomycorrhizal fungi resulted in greater leaf tissue P concentration compared to non-inoculated seedlings.

Table 1. Biomass distribution, leaf area, mycorrhizal colonization and nodule dry weight of *Leuceana leucocephala* seedlings inoculated with vesicular-arbuscular mycorrhizal and ectomycorrhizal fungi

Fungal symbiont	Total plant dry wt. (g)	Root dry wt. (g)	Leaf area (cm^2)	Mycorrhizal colonization (%)	Nodule dry wt. (mg)	Seedling P %
Gm[2]	12.0[a1]	5.6[ab]	569[a]	67[ab]	20.3[a]	0.21[a]
Gd	11.9[a]	5.9[a]	650[a]	38[b]	26.2[a]	0.21[a]
Gi	10.8[ab]	5.3[ab]	397[b]	91[a]	21.7[a]	0.22[a]
Ge	11.3[ab]	5.6[ab]	508[b]	64[ab]	20.8[a]	0.21[a]
Pt	10.1[b]	4.4[b]	548[ab]	-	17.1[ab]	0.17[a]
Ll	10.6[b]	5.0[ab]	550[ab]	-	26.6[a]	0.18[a]
Ni	8.6[c]	4.8[ab]	249[b]	-	10.1[b]	0.13[b]

[1] Means within a column followed by a common letter are not significantly different by LSD test (p= 0.05);

[2] Fungal symbiont abbreviations: *Gm = Gigaspora margarita, Gd = Glomus deserticola, Gi = G. intraradices, Ge = G. etunicatum, Pt = Pisolithus tinctorius, Ll = Laccaria laccata* and Ni = Non-inoculated.

Leaf water potential of the *Leucaena* seedlings varied significantly with mycorrhizal symbiont during the short-term drought (Table 2). Plants inoculated with *Gigaspora* and *Glomus* species maintained higher leaf water potential during the period of peak water stress relative to non-inoculated seedlings. In contrast, water potential of plants inoculated with ectomycorrhizal fungi was slightly greater than non-inoculated seedlings during peak water stress. Following re-watering of the growth medium, leaf water pressure potential of seedlings inoculated with VAM fungi recovered from water stress to pre-drought levels.

Leucaena leaf stomatal conductance did not always correspond with patterns of leaf water potential (Table 2). Plants inoculated with *Glomus* and *Gigaspora* species had greater rates of leaf conductance during pre-, peak-, and post-water stress periods relative to seedlings inoculated with ectomycorrhizal fungi. The non-inoculated seedlings exhibited relatively low leaf conductance but this response varied during the cyclic drought.

Photosynthesis rates of the *Leucaena* seedlings were significantly influenced by mycorrhizal symbiont and water stress (Table 2). Prior to water stress seedlings

inoculated with VAM and ectomycorrhizal fungi had significantly greater rates of photosynthesis compared to non-mycorrhizal plants. At peak stress seedlings inoculated with VAM fungi had greater rates of photosynthesis compared to non-mycorrhizal plants. After re-watering the growth medium, photosynthesis of VAM seedlings was significantly greater than non-mycorrhizal plants.

Table 2. Leaf water potential (Ψ), leaf stomatal conductance (K) and photosynthesis rate (Ps) of *Leucaena leucocephola* seedlings inoculated with VAM and ectomycorrhizal fungi at mid-light during pre-, peak- and post- water stress conditions

Fungal symbion Ψ	(Ψ) (*-MPa*)			(K) ($mmol\ m^{-2}s^{-1}$)			Ps ($\mu mol\ m^{-2}s^{-1}$)		
	pre-	peak-	post-	pre-	peak-	post-	pre-	peak-	post-
Gm[2]	0.5^{b1}	0.9^{c}	0.5^{c}	272^{a}	111^{d}	314^{a}	8^{a}	4^{b}	8^{a}
Gd	0.4^{c}	0.9^{c}	0.5^{c}	243^{ab}	123^{cd}	229^{c}	9^{a}	8^{a}	9^{a}
Gi	0.4^{c}	0.7^{c}	0.3^{d}	212^{bc}	177^{a}	191^{c}	7^{ab}	7^{a}	8^{a}
Ge	0.4^{c}	0.9^{c}	0.5^{c}	265^{a}	143^{b}	277^{b}	8^{a}	4^{b}	9^{a}
Pt	0.4^{c}	1.1^{b}	0.9^{b}	193^{cd}	132^{bc}	207^{c}	4^{c}	1^{c}	3^{b}
Ll	0.5^{b}	1.2^{b}	0.8^{b}	191^{cd}	127^{c}	218^{c}	6^{b}	1^{c}	2^{bc}
Ni	0.6^{a}	1.8^{a}	1.2^{a}	167^{d}	63^{e}	76^{d}	2^{d}	1^{c}	1^{c}

[1] Means within a column followed by a common letter are not significantly different by LSD test (p = 0.05);

[2] Fungal symbiont abbreviations: *Gm* = *Gigaspora margarita*, *Gd* = *Glomus deserticola*, *Gi* = *G. intraradices*, *Ge* = *G. etunicatum*, *Pt* = *Pisolithus tinctorius*, *Ll* = *Laccaria lacata* and Ni = Non-inoculated.

Discussion

Leucaena leucocephala seedlings inoculated with the four VAM species exhibited comparatively different degrees of root colonization. Habte and Fox (1992) and Huang *et al.* (1985) also observed that the ability of *L. leucocephala* to form endomycorrhizae was dependent on the fungal isolates. Similar responses to VAM fungi have been observed in the tree genera *Citrus* (Menge *et al.* 1978), *Juglans* (Dixon 1988) and *Liquidambar* (Kormanik 1981). The VAM isolates used in this study were all from the same geographic origin (Delhi, India) but differences in mycorrhizal colonization between species were significant. Inoculation with the ectomycorrhizal fungi *Laccaria* and *Pisolithus* did not result in the formation of Hartig net but a mantle of hyphae was observed. Pseudo-ectomycorrhizal and ectomycorrhizal structures of *L. leucocephala* have been reported previously (Rao *et al.* 1989,Osonubi *et al.* 1991). The geographic origin of the ectomycorrhizal fungi seemed to have little influence on seedling mycorrhizal symbiosis in this study.

Inoculation of *L. leucocephala* with VAM fungi stimulated seedling shoot and root dry weight and leaf area, whereas the presence of ectomycorrhizal fungi did not significantly influence plant morphology. The morphological response of *L. leucocephala* was VAM species specific. Michelsen and Rosendahl (1990) and Habte and Fox (1992) observed that juvenile biomass accretion of *L. leucocephala* was enhanced by the presence of VAM fungi or adequate supply of soil P.

Drought reduced biomass accretion of VAM *L. leucocephala* seedlings in earlier short-term studies (Michelsen & Rosendahl 1990, Osonubi *et al.* 1991) but this experiment was not designed to measure this response.

Earlier assessments revealed that the legume *L. leucocephala* formed ectomycorrhizae and endomycorrhizae. Although genuine ectomycorrhizae were not observed on the root systems of *L. leucocephala* seedlings, ectomycorrhizae-like structures were observed (Rao *et al.* 1989, Osonubi *et al.* 1991) and their presence stimulated nodulation by *Rhizobium* (Table 1). Nodulation of *L. leucocephala* by *Rhizobium* was also associated with seedlings having abundant VAM colonization. The interdependent tri-partite relationship of the plant host, *Rhizobium* and mycorrhizal fungi has been demonstrated in annual legumes (Bethenfalvay & Yoder 1981).

Significantly greater leaf tissue P content of *L. leucocephala* colonized with VAM was observed in earlier studies (Michelsen & Rosendahl 1990). Differential *Leucaena* nutrition response to VAM fungal species was also reported by Bagyaraj *et al.* (1989) as isolates from various geographic origins were compared. Bethenfalvay and Yoder (1981) and Menge *et al.* (1978) observed that differential P nutrition in *Citrus* was closely associated with VAM species and their ability to exploit soil for poorly mobile anions and cations (Harley & Smith 1983). The accumulation of polyphosphate (Strullu 1983) in VAM structures may contribute to root water transport or seedling capability for osmotic adjustment during water stress (Nelsen 1987).

Earlier assessments revealed that ectomycorrhizal symbiosis can increase root water uptake (Dixon *et al.* 1980, Duddridge *et al.* 1980), reduce leaf water potential (Parke *et al.* 1983, Dixon & Hiol Hiol 1992) and enhance transpiration flux and stomatal conductance (Lamhamedi *et al.* 1992). However, the water stress response of VAM plants is not necessarily similar to ectomycorrhizal plants and drought avoidance or tolerance mechanisms vary (Nelsen 1987). In this study, *L. leucocephala* with abundant VAM resulted in leaf xylem pressure potential and conductance rates greater than the non-mycorrhizal control seedlings at the peak of drought, even though VAM seedlings were significantly larger in biomass and leaf area. Leaf conductance of VAM seedlings was greater than control plants following re-watering and recovery from drought stress. It appears that VAM mycelial strands can transport physiologically significant quantities of water from the bulk soil to the root-fungus interface (Nelsen 1987). Differences in *L. leucocephala* leaf water potential and stomatal conductance among VAM species may be a result of differential mycelia distribution in soil or variation in mycorrhizae morphology such as contact of vesicles and arbuscules with the plasmalemma of root cortical cells (Huang *et al.* 1985, Nelsen 1987).

Seedlings inoculated with VAM fungal species exhibited photosynthesis rates greater than non-mycorrhizal control plants under well-watered and drought stress conditions. Photosynthesis of mycorrhizal plants is generally greater than non-mycorrhizal seedlings under well-watered conditions (Dosskey *et al.* 1990, Lamhamedi *et al.* 1992). This phenomenon has been attributed to greater root sink strength for photosynthate (Ekwebelam & Reid 1983, Dosskey *et al.* 1990), im-

proved nutrition, especially P (Nelsen 1987), and ecophysiological changes in leaves (Parke *et al.* 1983, Auge *et al.* 1986). Huang *et al.* (1986) observed that leaf orientation of mycorrhizal *L. leucocephala* seedlings may favor photosynthesis and assimilate transport to other organs. Recently, it was observed that variability in photosynthesis of Douglas-fir (*Pseudotsuga menziessii*) was closely correlated with the isolate of the ectomycorrhizal symbiont (Dosskey *et al.* 1990). The results of this study support the hypothesis that fungal genotype influences patterns of photosynthesis in the host plant.

In addition to the possible morphological structures of VAM which enhance root water uptake and/or transport, the *Glomus* and *Gigaspora* species may have also influenced metabolic activity in leaves. The synthesis and transport of cytokinin, abscisic acid and proline in mycorrhizal plants have been linked to stomatal regulation (Levy & Krikun 1980, Auge *et al.* 1986, Dixon *et al.* 1988, Coleman *et al.* 1990, Zhang & Davies 1990). Greater rates of transpiration and photosynthesis may be associated with osmotic adjustment of leaf water potential (Auge *et al.* 1986) or high cytokinin/abscisic acid ratios in guard cells (Dixon *et al.* 1988) during periods of water stress. Mimosine content of *L. leucocephala* increases with leaf age and water stress (Bray & Hoekstra 1985). Many factors influence stomata aperture and transpiration and the complex role of VAM symbionts is not fully known.

Leucaena leucocephala is among the most widely distributed fast-growing nitrogen-fixing tree species within the humid and semi-arid tropics (Felker *et al.* 1983, Habte & Fox 1992). The ability of *Leucaena* to adapt to an extremely wide range of edaphic and climatic conditions may be due, in part, to its symbiotic partners. Vesicular-arbuscular mycorrhizal fungi provide a number of ecophysiological benefits to *Leucaena* including expansion of the root-fungus absorbing area to help the host avoid drought (Huang *et al.* 1985). The range of responses to cyclic drought exhibited by the *Leucaena* x mycorrhizal fungi combinations in this study support this hypothesis.

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