

# Humid tropical leguminous tree and pasture grass responsiveness to vesicular-arbuscular mycorrhizal infection

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**Abstract.** Phosphorus is the major nutrient limiting plant growth in a Costa Rican silvopastoral system located on an acid, high P-retaining, volcanic soil. We investigated plant responsiveness to vesicular-arbuscular mycorrhizal (VAM) inoculation using the leguminous tree species *Erythrina berteroana* Urban, and the two dominant grass species *Paspalum conjugatum* Berg and *Homolepsis aturensis* Chase of this silvopastoral system. We grew grass seedlings in the greenhouse for 15 weeks in a methyl bromide-sterilized study soil to which either mixed-species VAM inoculum (*Theobroma cacao* feeder roots) or autoclave-sterilized cacao roots (non-inoculated control) were added. *E. berteroana* was grown from both seedlings and vegetative stakes (40 cm long) for 30 and 19 weeks, respectively. Upon harvest, we measured above and below ground biomass, N and P content, root:shoot ratio, legume nodulation, and VAM infection levels. The total above-ground and root biomass of mycorrhizae-inoculated *P. conjugatum* seedlings were 2.5 and 2.8 times greater than those of noninoculated seedlings. In contrast, VAM-inoculated seedlings of *H. aturensis* produced 8.4 and 5.9 times more total above-ground and root mass than noninoculated seedlings. Mycorrhizae-inoculated *E. berteroana* seedlings produced 10.6 times greater shoot biomass for inoculated versus noninoculated seedlings, while *E. berteroana* vegetative stakes exhibited a negative growth response to VAM inoculation (an approximately 16% decrease in shoot biomass for VAM-inoculated cuttings). The difference in responsiveness between *Erythrina* growth forms is hypothesized to reflect the cost-benefit relationship between plant host and fungal symbiont for energy and nutrient reserves.

**Key words:** Silvopastoral system – Costa Rica – *Erythrina berteroana* Urban – *Paspalum conjugatum* Berg – *Homolepsis aturensis* Chase

## Introduction

Few would dispute that low phosphorus availability is a major constraint to pasture productivity on soils of the neotropics (Jehne 1980; Janos 1983; Sieverding and Saif 1984; Ae et al. 1990). Although volcanic soils and those with Fe and Al oxides contain large amounts of total P, the majority exists as stable organic and inorganic mineral-bound complexes, leaving little in plant-available form. Consequently, many plant species native to soils with high P retention capacities possess mechanisms to access a range of soil P pools. The majority of these mechanisms are biologically mediated, the most notable among them being vesicular-arbuscular mycorrhizae (VAM) (Salinas and Sanchez 1976; Jehne 1980; Janos 1988).

Numerous studies have shown that the extent of VAM effectiveness (plant host responsiveness) depends upon both plant and associated fungus species and is inversely related to available soil P (Yost and Fox 1979; Stribley et al. 1980; Abbott and Robson 1987; Huang and Yost 1987; Siqueira 1987). Plant responsiveness is considered an extrinsic characteristic of the host-fungus relationship and is usually evaluated as relative plant growth with and without mycorrhizae (Janos 1988, 1993). Host-VAM dependency, in contrast, is an innate plant property and relates to the level of soil fertility (e.g., soil P) below which plants do not grow without mycorrhizae (Janos 1993). Janos (1980, 1983) proposed a continuum of VAM dependency for tropical plants ranging from facultative to obligate mycotrophy. For example, cool season or C<sub>3</sub> grasses have been shown to be less mycorrhiza dependent than warm season C<sub>4</sub> grasses, and nitrogen-fixing legumes are highly mycorrhizal dependent relative to grasses (Sieverding and Saif 1984; Hetrick et al. 1988; Wilson et al. 1991).

From an agricultural perspective, combining plants with different VAM dependencies or responsiveness to VAM infection could be used to reduce fertilizer inputs on nutrient-poor soils (Salinas et al. 1985). Sieverding and Saif (1984) showed that herbaceous leg-

umes that were VAM-inoculated in the field with only 20 kg P/ha fertilizer added produced 68% more biomass than noninoculated plants after 3 months growth. Likewise, Siqueira (1987) reduced the P requirement for *Stylosanthes* (an herbaceous forage legume) by 97% with VAM inoculation. Huang et al. (1985), working with the leguminous tree *Leucaena leucocephala*, obtained four times taller plants and 80 times greater dry stem mass with mycorrhizal specimens than with nonmycorrhizal specimens.

Mycorrhiza-infected legumes also have the potential to supply additional nutrients like N and energy (C) to neighboring plants. Several studies suggest that some species within plant communities are interlinked via mycorrhizal hyphae and that mycorrhizae facilitate transfer of C and nutrients among plants (Perry et al. 1992). Francis and Read (1984), Chiariello et al. (1982) and Fischer et al. (1993) have demonstrated direct transfer of C and nutrients through mycorrhizal hyphae linkages among grass species. Alternatively, close association between legumes and grasses may enhance recipient uptake of nutrients by either donor leakage or diminished soil N uptake by the legume (Danso et al. 1993). Danso and coworkers found that N content and dry matter yield of *Avena sativa* intercropped with *Lupinus angustifolius* were enhanced 26% and 21%, respectively, relative to monocropped *A. sativa*. Van Kessel et al. (1985) proposed that VAM-mediated nutrient transfer from a legume (*Glycine max*) to a non-legume (*Zea mays*) resulted in significantly higher leaf and root N in VAM-inoculated relative to noninoculated maize. In either case, strongly mycotrophic legumes have the potential to increase neighboring plant productivity on soils with inherently low nutrients.

Assuming that legumes are more mycotrophic than grasses, one could argue that under low available soil P conditions VAM-infected legumes would be better scavengers for P than grasses and, therefore, stronger P bioaccumulators (Krikun 1991). Accordingly, VAM-infected legumes should enhance P cycling when planted in association with less mycotrophic pasture grasses (Ae et al. 1990; Perry et al. 1992). The general objective of this present study was to evaluate the role of VAM in both leguminous tree and pasture grass P uptake and biomass production in a humid tropical silvopastoral system. Specifically, we sought (1) assess inherent levels of VAM infection in native grass pastures of the Costa Rican Atlantic coastal region, and (2) determine plant responsiveness to VAM inoculation using biomass and nutrient uptake parameters for the two dominant grass species (*Paspalum conjugatum* Berg and *Homolepsis aturensis* Chase) and the leguminous tree *Erythrina berteroana* Urban.

## Materials and methods

### Study site

The study site was located on the Atlantic coastal plain of Costa Rica in Limon Province (10° N 83° W; 30–50 m above sea level). The area receives 3630 mm rainfall annually, most of which is

distributed evenly throughout the year. The Holdridge ecological lifezone is lowland humid tropical rainforest, although the settlement in which the farms were located was deforested for the most part over 20 years ago. The study soil was classified according to US Soil Taxonomy as an Andic Humitropept, series Neguev, and was derived from volcanic debris and mudflows from the late Pleistocene. Due to its stable position on the landscape and high rainfall, the Neguev soil has undergone intense weathering and, accordingly, possesses low pH (4.8–5.1) low CEC (~2.6 cmol/kg pH 4.8 NH<sub>4</sub>-acetate extracted) and a very high P retention capacity (>2000 mg P/kg soil). The field experiment, a silvopastoral system, included cattle grazing and presence or absence of *E. berteroana* as the two main effects (a 2×2 factorial design). Blocks of the four treatments were established on five farms in 1987.

### Field and laboratory methods

To determine the level of VAM infection in native pastures, we collected 18 root samples per farm by cutting pieces of turf (30 cm × 30 cm × 10 cm), separating out fine feeder roots and placing roots directly into vials containing formalin-acetic acid-alcohol solution. After removing soil from roots, we placed a subsample from each vial in Omnisette tissue microcassettes for clearing and staining.

For the greenhouse experiments, we collected seeds of *E. berteroana* and the two principal grass species, *H. aturensis* and *P. conjugatum* from the field study farms. Seeds were germinated in nonsterilized vermiculite. A composite soil sample from all five farms (approximately 40 kg of unamended field-moist soil) was sterilized with methyl bromide.

Soil chemical properties were altered by fumigation with methyl bromide (Table 1). When compared with values obtained for nonfumigated field soil, fumigated soil pH was 0.2–0.8 units lower, NaHCO<sub>3</sub>-EDTA extractable P was three times higher, NaHCO<sub>3</sub>-EDTA extractable Zn 16 times lower and NaHCO<sub>3</sub>-EDTA extractable Mn 36 times higher. Exchangeable bases, acidity and NaHCO<sub>3</sub>-EDTA extractable Cu were not affected by fumigation. Other studies have reported side effects of sterilization on soil chemistry including enhanced P availability and dramatic increases in extractable Mn (Williams-Linera and Ewel 1984; Borie 1985; Stribley 1987). Manganese levels may have been high enough to impart plant toxicity; however, treatment differences were still maintained.

Approximately 1 month after seeds were planted, we transplanted seedlings to individual pots (one plant per pot) containing approximately 700–800 g field-moist, sterilized soil. For each

**Table 1.** Chemical characteristics of the study soil, series Neguev, before and after fumigation with methyl bromide. All NaHCO<sub>3</sub>-EDTA extractable elements are expressed as μg/g soil. All exchangeable elements were determined via NH<sub>4</sub>OAc-pH 7 extraction and expressed as cmol/kg soil

Soil chemical property	Non-fumigated soil	Fumigated soil	Level of significance ( <i>P</i> value)
pH in H <sub>2</sub> O <sup>a</sup>	4.7–4.9	4.4–4.5	0.0001
NaHCO <sub>3</sub> -extractable P	7.98	17.03	0.027
NaHCO <sub>3</sub> -extractable Cu	26.01	24.00	ns
NaHCO <sub>3</sub> -extractable Zn	66.94	4.09	0.027
NaHCO <sub>3</sub> -extractable Mn	4.12	142.63	0.0001
Exchangeable K	0.33	0.27	ns
Exchangeable Ca	0.92	1.16	ns
Exchangeable Mg	0.83	1.24	0.004
Exchangeable acidity	2.05	1.87	ns

<sup>a</sup> Determined as 1:1 solution

species there were seven replicates per treatment, except for *Homolepsis* ( $n=6$ ). On the same day seedlings were transplanted, we added the VAM cacao feeder root inoculum which was prepared according to Janos (1984). A subsample of the inoculum was autoclaved to serve as the control. In addition, we added a non-VAM microorganism solution prepared by soaking cacao feeder roots in deionized water and filtering the liquid through Whatman No. 1 paper. All pots received a N, K nutrient solution 4 and 18 days after planting (50 ml total volume, of which 25 ml was 25  $\mu\text{g/ml}$  N and 25 ml was 20  $\mu\text{g/ml}$  K). Seedlings were watered with tap water as needed to maintain relatively constant soil moisture.

At the end of 15 weeks, we harvested the two grass species for leaf, stem, and root dry weight biomass and root volume determinations. Biomass samples were dried and ground and foliar nutrient contents determined (N, P, Mg, Ca, K, Cu, Mn) in  $\text{HNO}_3\text{-HClO}_4$  acid digests (Briceño and Pacheco 1984). P content is the only nutrient reported for *Paspalum* (other elemental results are presented in Cooperband 1992). For *Homolepsis*, only those samples from the VAM-inoculated treatment had sufficient material for chemical analysis. As such, no nutrients are reported for *Homolepsis*.

Because of the woody phenology and lower relative growth rate of *Erythrina*, we allowed the seedlings to grow longer than the grasses before harvesting (30 weeks). We also repeated the experiment using *Erythrina* grown from vegetative cuttings, because *Erythrina* is rarely planted from seed in the field. We cut 40-cm-long stakes from a single *E. berteroana* tree and planted them in sterilized soil with and without inoculum as described above (pot size: 24 cm diameter, 22 cm tall). Stakes were harvested after 19 weeks from planting.

Parameters measured for *Erythrina* stakes included leaf and regrowth stem biomass and foliar chemical analysis, while *Erythrina* seedling measurements included these plus root biomass and the presence of nodules. Nodules were evaluated using a qualitative index which included abundance, size and color (red indicates nodules are actively fixing N): 1, absent; 2, present and few; 3, present, numerous and red; and 4, present, abundant, large and red. Biomass samples were analyzed for N, P, macro and micro nutrients. The noninoculated seedling material was composited and analyzed as a single sample due to low biomass.

We cleared and stained both field and greenhouse roots for percent VAM infection according to Koske and Gemma (1989). Percent infection and total root length was determined for field-collected roots using the grid-intersection method of Giovanetti and Mosse (1980), whereas greenhouse roots were evaluated using the slide-intersection method (Biermann and Linderman 1981; Sieverding 1983). Roots from each plant were cut into small segments, and 5–10 of these segments were placed on a slide for scoring. Percent infection was determined by scanning the stage across the slide and counting the number of intersections with VAM structures from a total of 80 visuals (in this case, either internal hyphae or vesicles; no arbuscules were encountered).

While scoring greenhouse roots for percent intersections infected with VAM, we noted the percent of infected intersections with vesicles and hyphae separately, because roots infected with hyphae alone or with hyphae and vesicles may represent infection from different VAM families. Percent total and the proportion of vesicle to total infection were evaluated for the two grass species, *Erythrina* seedlings and *Erythrina* cuttings. The cacao feeder root inoculum was also collected from several inoculated and control treatments upon plant harvesting and roots were evaluated for percent infection. Total infection in cacao inoculum ranged from 15 to 63%, while percent hyphal with vesicle infection ranged from 14 to 65%.

One-way ANOVAs were performed by species to assess the effect of inoculation on total infection levels as well as the ratio of vesicle to total infection (SYSTAT MGLH procedure for analysis of variance; Wilkinson 1990). Percent total infection was arc-sine-square root transformed and the vesicle:total infection ratio

was square root transformed prior to statistical analysis. In addition, we performed ANOVAs by species for the effect of inoculation on each biomass (total plant mass, root mass, shoot mass, root:shoot ratio, root volume) and nutrient (total N, P) parameter.

## Results

VAM infection levels of the native grass pastures ranged from 67 to 76% of total root length (Table 2). Among-farm differences in infection level were not significant. These levels were consistent with those reported for tropical pasture species (Sieverding and Saif 1984; Berbara et al. 1985).

Mycorrhizal infection levels for all plants in the greenhouse study were, on average, significantly lower than field infection levels (71% versus 47% average infection for field and greenhouse studies, respectively). However, this is a common by product of greenhouse inoculation experiments (Mosse 1977; Borie 1985; Kollowsky and Boerner 1989). Nonetheless, inoculation significantly increased total infection levels for all species except *H. aturensis* (Table 3). Inoculated *P. conjugatum* plants were three times more infected than noninoculated, while *E. berteroana* grown from seedlings and cuttings were ten and three times more infected, respectively, than noninoculated plants.

While there was some VAM infection in noninoculated plants, only inoculated plants possessed vesicles. *P. conjugatum* was the exception, however, with 24% of infected noninoculated roots containing vesicles. Although infection levels in noninoculated *H. aturensis* were high, no vesicles were observed in noninoculated roots. In contrast, 90% of infected inoculated *H. aturensis* plants had vesicles. In addition, when evaluated on a per root weight basis (% in infection per gram of roots), less than 4% of root mass of noninoculated plants contained VAM structures. In an absolute sense, then, VAM infection of noninoculated *H. aturensis* plants was sparse.

In terms of plant growth and nutrient uptake, *H. aturensis* and *P. conjugatum* responded significantly to VAM inoculation (Table 4). Although the levels of significance for the different growth parameters were lower for *Homolepsis* relative to *Paspalum*, the relative responsiveness to VAM infection of *Homolepsis* was actually more pronounced than that of *Paspalum*. To-

**Table 2.** Inherent pasture vesicular-arbuscular mycorrhizae (VAM) infection levels measured from farm field samples. The number of samples per farm was 18. The differences among the farm samples are not significant

Farm	Mean VAM infection (%)	Standard error
1	72.57	2.39
2	69.14	1.84
3	69.05	2.28
4	75.51	2.57
5	66.93	2.65

**Table 3.** Total VAM infection levels and the proportion of vesicle to total infection for inoculated and noninoculated grass species and *Erythrina berteroana* from seedlings and cuttings. The ratios were calculated from the number of sites infected with ves-

icles divided by the total number infected. Where two numbers of plants sampled are given, the first number is noninoculated plants and the second is inoculated plants. Numbers in parentheses are standard errors

Plant species	Number of plants sampled	Total VAM infection (%)			Ratio of vesicles: total infection		
		Non-inoculated	Inoculated		Non-inoculated	Inoculated	
<i>Paspalum conjugatum</i>	6/7	18.1 (4.0)	55.8 (5.4)	***	0.24 (0.12)	0.49 (0.11)	ns
<i>Homolepsis aturensis</i>	6	62.0 (5.9)	57.5 (8.7)	ns	0.0 (0.0)	0.90 (0.09)	***
<i>Erythrina berteroana</i> (seedlings)	4/7	4.2 (4.2)	41.4 (3.0)	***	0.0 (0.0)	0.25 (0.03)	**
<i>Erythrina berteroana</i> (cuttings)	5	12.1 (7.2)	32.8 (3.4)	*	0.0 (0.0)	0.20 (0.07)	ns

\*  $P < 0.05$ , \*\*  $P < 0.001$ , \*\*\*  $P < 0.0001$

**Table 4.** Mean biomass (g) and P content (mg) of the two grass species examined. Total P of *Homolepsis* was not measured due to shortage of material. Numbers in parentheses are standard errors

Parameter	<i>Paspalum conjugatum</i>			<i>Homolepsis aturensis</i>		
	Noninoculated (n=6)	Inoculated (n=7)		Noninoculated (n=6)	Inoculated (n=6)	
Shoot mass	2.04 (0.38)	5.04 (0.69)	**	0.09 (0.03)	0.94 (0.37)	*
Root mass	0.61 (0.09)	1.70 (0.26)	**	0.07 (0.03)	0.41 (0.15)	*
Total mass	2.65 (0.46)	6.73 (0.92)	**	0.16 (0.06)	1.35 (0.52)	*
Total P	2.53 (0.36)	6.63 (0.73)	***	—	—	
R:S ratio	0.31 (0.02)	0.34 (0.03)	ns	0.83 (0.17)	0.75 (0.33)	ns
Root volume (cm <sup>3</sup> )	3.00 (0.43)	10.17 (1.72)	**	1.07 (0.29)	2.70 (0.58)	*

\*  $P < 0.05$ ; \*\*  $P < 0.002$ ; \*\*\*  $P < 0.0001$

tal plant mass of inoculated *Homolepsis* plants was approximately eight times greater and root volume was almost three times greater than noninoculated plants. In contrast, *Paspalum* biomass was enhanced more than twofold by inoculation. Root volume of inoculated *Paspalum* was approximately 30% greater than noninoculated plants. There was also a strong mycorrhizal infection effect on total P content of *Paspalum* (6.63 mg/inoculated plant versus 2.53 mg/noninoculated plant). The root:shoot ratio did not differ significantly between inoculated and noninoculated plants of either species, suggesting that the relative growth of above- and below-ground biomass was not affected by VAM infection.

Mycorrhizal infection had a strong positive effect on *E. berteroana* seedling growth and nutrient uptake, while the effect was either neutral or negative for *E. berteroana* grown from vegetative cuttings (Table 5). Total seedling mass and total P content for inoculated seedlings were nine times greater and total N content was five times greater than noninoculated plants. The root:shoot ratio was not affected by inoculation, as was found for the two grass species. In contrast to *E. berteroana* grown from seed, new growth biomass and P uptake for *E. berteroana* grown from cuttings were negatively affected by mycorrhizal inoculation ( $P < 0.05$ ). New growth mass for noninoculated stakes was 1.2 times higher than inoculated stakes (7.0 versus

5.9 g) and total new growth P was similar (13.9 versus 10.3 mg P/plant).

Mycorrhizal infection also had a significant effect on nodulation of *E. berteroana* seedlings. According to the qualitative index used to assess nodulation, 100% of the noninoculated seedlings produced no nodules after 30 weeks (index=1), whereas 98% of all inoculated seedlings had either numerous or abundant large red nodules (index=3 and 4). In addition, none of the *E. berteroana* stakes produced nodules after 18 weeks.

## Discussion

The presence of some VAM infection in noninoculated plants suggests that sterilization via methyl bromide did not kill all of the mycorrhizal spores present in the study soil or that spores were transported between inoculated and noninoculated pots (S. Rabbatin, Ricera Inc., unpublished work). However, the nearly complete absence of vesicles in noninoculated plant roots suggests that different VAM families were present in the cacao feeder root inoculum than in the field soil. Several studies suggest that different VAM communities dominate tropical agro-ecosystems and forests in different successional stages (Rose and Paranka 1987; Cuenca and Lovera 1992; Cuenca 1993). Both Cuenca (1993) and Janos (1975) found that *Acaulospora* and

**Table 5.** Mean biomasses (g) and nutrient contents (mg) of VAM inoculated and noninoculated *Erythrina berteroana* grown from both seedlings and vegetative cuttings. The root mass of vegeta-

tive cuttings was not determined due to shortage of material. Numbers in parentheses are standard errors

Parameter	Seedlings			Vegetative cuttings	
	Noninoculated (n=4)	Inoculated (n=7)		Noninoculated (n=5)	Inoculated (n=5)
Leaf mass	0.04 (0.01)	0.70 (0.22)	*	3.16 (0.15)	2.88 (0.23)
Stem mass	0.16 (0.03)	1.42 (0.33)	*	3.84 (0.29)	3.03 (0.27)
Shoot mass	0.20 (0.03)	2.12 (0.55)	*	7.00 (0.20)	5.91 (0.37)
Root mass	0.13 (0.03)	0.84 (0.13)	**	—	—
R:S ratio	0.66 (0.16)	0.46 (0.06)	ns	—	—
Total mass	0.33 (0.06)	2.96 (0.95)	*	—	—
Total P	0.73 (0.13)	6.58 (1.15)	**	13.89 (1.15)	10.27 (0.98)
Total N	34.18 (6.79)	174.95 (41.8)	*	768.02 (57.0)	660.43 (95.4)

\*  $P < 0.05$ , \*\*  $P < 0.01$

*Glomus* are the two dominant genera in cacao plantations. Both genera, which fall within the Glomineae family, are known to form intraradical vesicles. Areas infected without vesicles may have come from pasture soil-borne spores, and may represent VAM species that produce only extraradical vesicles or auxilliary cells, e.g., those from the family Gigasporaceae. It was impossible to verify this hypothesis since spore identification was not within the scope of this study. Nonetheless, given the considerable growth period prior to harvest, it is likely that plants without vesicles after 15 weeks would not have produced vesicles at some later point in their growth (J. Morton, Virginia Polytechnical Institute, unpublished work).

Although both grasses were responsive to VAM infection, the magnitudes of their responsiveness differed. Such differences may be related to infection by different VAM families and/or differences in host plant-VAM species effectiveness. Numerous studies support the hypothesis that VAM species-host plant associations differ in their effectiveness, i.e., certain mycorrhizae species produce large, positive growth effects with certain plants and less significant or even deleterious effects with other plant species (Fitter 1977; Sieverding and Toro 1987; Carling and Brown 1980; Boerner 1990, 1992; Perry et al. 1992). Furthermore, others suggest that effectiveness can vary as a function of host-fungus ontogeny and changes in external soil chemical and physical conditions (Bethlenfalvay et al. 1987; Rosendahl et al. 1990; Sieverding 1990; Collins Johnson et al. 1992).

Interspecific differences in VAM effectiveness-host plant responsiveness also evoke speculation that the two grasses are located at different points along the facultative-obligate mycotrophic continuum (Janos 1980, 1983). *Homolepsis* appeared to be more responsive to VAM inoculation at the low level of P availability inherent to the study soil and, therefore, less facultative than *Paspalum*. Additionally, noninoculated *Paspalum* had less VAM infection than *Homolepsis*, and yet grew more relative to noninoculated *Homolepsis*; this further suggests that *Paspalum* is the more facultative of the two grasses. Regardless, both grasses

were strongly responsive to VAM infection. Moreover, the greenhouse data corroborate findings in the field and confirm the importance of the VAM-host plant relationship in soils with inherently low bioavailable P.

*E. berteroana* seedlings were also highly responsive to inoculation with VAM. In addition, nodulation was strongly linked to mycorrhizal infection. These results suggest that, like most N-fixing legumes, *E. berteroana* is an obligate mycotroph (Janos 1980; Krikun 1991). With regard to responsiveness to VAM infection and forage quality, studies comparing forage legumes with grasses have demonstrated that legumes produce larger responses to VAM infection in terms of both biomass production and nutrient uptake (Bolan et al. 1987; Siqueira 1987). If certain host-VAM associations result in enhanced nutrient uptake, particularly P (e.g., Boerner 1990), the relationship could be viewed as a means to improve forage quality. In the case of combining leguminous trees with pasture, the increased *Erythrina* biomass and foliar P levels support the hypothesis that mycorrhizae are important for enhanced P cycling in the silvopastoral system. Since VAM infection results in greater *Erythrina* leaf P contents, more P should be released into the available soil P pool as leaves decompose and P is mineralized. In addition, improved nutrient status could augment resilience to grazing and enhance resistance to disease (Jehne 1980; Wallace 1981).

The difference in growth response between VAM-inoculated *E. berteroana* seedlings and vegetative cuttings suggests a somewhat plastic relationship between plant host and fungal symbiont. For seedling establishment, initial root growth is critical for nutrient uptake because seed and cotyledon reserves are limited. If association with VAM fungi at an early stage promotes root growth and subsequent nutrient uptake, the ensuing positive feedback mechanism confers an advantage (increased growth) over seedlings with no VAM association (Huang et al. 1985; Habte and Manjunath 1987; Huang and Yost 1987). Several studies allude to the synergistic relationship between *Rhizobium* and VAM (Mosse 1977; Barea and Azcon-Aguilar 1983; Sieverding and Saif 1984). Most confirm that VAM infection

stimulates nodulation (van Kessel et al. 1985; Wadsirirak et al. 1987). For *Erythrina* seedlings, then, the VAM-associated benefits of root establishment and nodulation outweighed the cost associated with maintaining the VAM fungal symbiont.

In contrast, establishment and growth of *Erythrina* vegetative cuttings relied on a different nutrient and carbon acquisition strategy. The negative effect incurred from VAM inoculation suggests that, in the initial phase of stake establishment, the cost of C drain to support the mycorrhizal symbiont outweighed the potential benefit of enhanced nutrient uptake from the plant's association with the fungus (Buwalda and Goh 1982; Clarkson 1985; Bethlenfalvay et al. 1987). During this early stage, the stake's ability to generate both new above-ground growth and roots was a function of stake size, i.e., nutrient and energy storage capacity. Therefore, it relied primarily on internal reserves and not nutrient uptake from the soil for initial establishment and above-ground growth. Accordingly, any association with a C-demanding symbiont would lead to competition for nutrients and energy and would diminish limited internal reserves (Eissenstat et al. 1993; Peng et al. 1993). The absence of nodules on both noninoculated and VAM-inoculated stakes further confirms the hypothesis that stake nutrient and C reserves were insufficient to support either VAM fungi or *Rhizobium* symbionts.

In conclusion, the balance between costs and benefits associated with the mycorrhizal symbiont shifts as a function of the plant host's internal nutrient and energy reserves. The evolution of this relationship should be investigated relative to tree establishment in agroforestry systems since *Erythrina* (as well as many other leguminous trees) is planted almost exclusively from vegetative stakes. The negative VAM effect may be an artifact of greenhouse establishment, and is probably transient in the field. It would be important to evaluate infection effects over a longer time period, both in the greenhouse and in the field, to assess the dynamic nature of the cost/benefit relationship.

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