

Post-transplant reactions of mycorrhizal and mycorrhiza-free seedlings of *Leucaena leucocephala* to pH changes in an Oxisol and Ultisol of Hawaii

M. Habte, G. Diarra, and P.G. Scowcroft

Abstract: The extent to which pretransplant colonization of seedlings with the arbuscular mycorrhizal fungus (AMF) *Glomus aggregatum* Schenck and Smith emend. Koske could enhance the post-transplant growth of two cultivars of *Leucaena leucocephala* (Lam.) de Wit (cv. K-8 and cv. K-636) in Al- and Mn-rich acid soils was evaluated in a greenhouse. Arbuscular mycorrhizal colonization measured at the end of the experiment was significantly stimulated by inoculation in both cultivars at all pH levels tested, although colonization was most stimulated if cv. K-8 was grown in the Al-rich soil at the lowest pH. Symbiotic effectiveness measured as P content of *Leucaena* pinnules was significantly suppressed in both cultivars if they were grown at the lowest pH. Symbiotic effectiveness measured as pinnule P content and shoot biomass yield was enhanced in both cultivars by liming. The trends in effectiveness were similar in both cultivars, but cultivar effect was significant in the Mn-rich Oxisol (Wahaiawa soil) but not in the Al-rich Ultisol (Leilehua soil). The tolerance of the cultivars to acid soil toxicity in the Wahaiawa soil varied with the pretransplant mycorrhizal status of their seedlings. The effect of pretransplant colonization of seedlings was to eliminate the differences in the tolerance of the cultivars to acid soil toxicity. Our data suggest that AMF could offset some of the growth reduction associated with soil acidity and that host genotype could play a role in this regard.

Key words: aluminum, cultivar, *Glomus aggregatum*, manganese, soil acidity.

Résumé : Les auteurs ont évalué en serre, jusqu'à quel point la précolonisation avant transplantation des plantules avec le champignon mycorrhizien arbusculaire (CMA) *Glomus aggregatum* Schenck and Smith emend. Koske peut favoriser la croissance post transplantation de deux cultivars du *Leucaena leucocephala* (Lam.) de Wit (cv. K-8 et cv. K-636) dans des sols acides, riches en Mn et Al. Chez les deux cultivars, la colonisation mycorrhizienne arbusculaire telle que mesurée à la fin de l'expérience, s'est vue stimulée à tous les degrés de pH testés, bien que la colonisation n'ait pas été stimulée lorsque le cv. K-8 a été cultivé dans un sol riche en Al au pH le plus bas. Chez les deux cultivars, l'efficacité de la symbiose, telle que mesurée par la teneur en P des pinnules du *Leucaena*, se voit supprimée lorsqu'on les cultive aux pH les plus bas. Chez les deux cultivars également, l'efficacité de la symbiose, telle que mesurée par la teneur en P des pinnules et le rendement en biomasse de la tige, s'avère stimulée par le chaulage. Les tendances de l'efficacité sont semblables chez les deux cultivars, mais l'effet du cultivar est significatif dans l'Oxisol riche en Mn (sol de Wahaiawa) mais pas dans l'Ultisol riche en Al (sol de Leilehua). La tolérance des cultivars à la toxicité des sols acides dans le sol de Wahaiawa varie avec le statut mycorrhizien avant transplantation des plantules. L'effet de la colonisation avant transplantation des plantules consiste à éliminer les différences de tolérance des cultivars à l'acidité toxique des sols. Les données suggèrent que les CMA pourraient contrebalancer la réduction de croissance associée à l'acidité des sols et que le génotype de l'hôte pourrait jouer un rôle à cet égard.

Mots-clés : aluminium, cultivar, *Glomus aggregatum*, manganèse, acidité du sol.

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Introduction

Soil acidity limits plant productivity worldwide, particularly in soils that have developed under humid temperate, tropical, and subtropical climates (Habte 1994; Kochian et al. 2004). These soils are characterized by relatively high concentrations of H⁺ and pH values of 5.5 or lower. However, the main constraints to plant productivity and the productivity of other macro- and microorganisms in these soils are toxic levels of aluminum (Al³⁺), manganese (Mn²⁺), and deficiency of nutrients, such as calcium (Ca), magnesium (Mg), phosphorus (P), and molybdenum (Mo) (Habte 1994; Kochian et al. 2004).

The most common management practice used to overcome

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the adverse effects of soil acidity is to lime the soils. However, the cost of liming can be prohibitive in many parts of the tropics, and conventional liming is not effective in correcting subsoil (Kochian et al. 2004; Islam et al. 2004) and rhizosphere acidity (Hinsinger et al. 2003). Decreased rhizosphere pH is expected, especially if ammonium is the main source of nitrogen for host plants (Robson and Abbott 1989) and in legume-based cropping systems (Bolan et al. 1991; Tang et al. 1999; Hinsinger et al. 2003). Alternative approaches are sought under these circumstances. One of these alternative approaches is the use of plant species and cultivars that are tolerant to soil acidity (Islam et al. 2004). Interest is also growing in the possibility of utilizing arbuscular mycorrhizal fungi (AMF) to minimize the effects of acid soil toxicities on associated plants (Yang and Goulart 1997; Cuenca et al. 2001; Lux and Cumming 2001). The objective of the current investigation was to evaluate the influence of the colonization of roots of seedlings of two cultivars of *Leucaena leucocephala* (Lam.) de Wit by AMF, prior to transplanting, on the growth and P uptake of the seedlings after they were transplanted in an Al-rich Ultisol and a Mn-rich Oxisol.

Materials and methods

Soils used

The soils used in the investigation were an Al-rich Ultisol (Leilehua series, oxidic, isothermic, and Typic Kandihumult) and a Mn-rich Oxisol (Wahiawa series, very fine, kaolinitic, isohyperthermic, and Kandjustalfic Utrustox). Surface soil samples (0–15 cm) were collected from the Waiawa Correctional Facility, Oahu, Hawaii (21°30'N; 157°50'W). Selected chemical properties of the soils are given in Table 1. The soils were screened by passing them through a 4.0 mm aperture sieve. Portions of the sieved soils weighing 2.25 kg were transferred into 15 cm × 15 cm plastic pots. The Leilehua soil had an initial pH (1:2 soil to water) of 4.8 and the Wahiawa soil had an initial pH of 5.1. The soils were either limed with dolomite or acidified with dilute sulfuric acid (0.25%) to obtain target pHs of 4.5, 5.0, and 6.2 for the Ultisol and 4.5, 5.5, and 6.4 for the Oxisol.

Raising seedlings and transplanting

The plant species used in the study were *L. leucocephala* cv. K-8 and cv. K-636. The two cultivars were selected for the study because they were presumed to be different in their response to acid soil toxicity. Seeds were scarified in concentrated sulfuric acid for 30 min, after which time they were rinsed four times in sterile water. The seeds were germinated on moistened sterile paper towels in Petri plates in the dark for 72 h. Meanwhile, UV-protected 164 mL dibble tubes (SC10 Super cell, Stuewe and Sons Inc., Tangent, Oregon) were filled with 70 g of Turface™ potting medium (calcined montmorillonite, Amicor Applied Industrial Material Corporation, Deerfield, Illinois). Dibble tubes are cone-shaped plastic cells for raising individual seedlings. They are wide at their mouth and tapered at their bottom. The holes at their bottom allow for pruning of roots by air. The dibble tubes that we used were 3.8 cm wide and 20 cm deep. Inoculation was performed by thoroughly mixing crude inoculum of *Glomus aggregatum* Schenck and Smith emend. Koske with Tur-

Table 1. Selected chemical properties of the experimental soils.

Soil	pH	mg·L ⁻¹	
		Al	Mn
Leilehua	4.5	173.0	12.0
	5.0	71.0	7.4
	6.2	19.0	7.5
Wahiawa	4.5	147.0	93.5
	5.5	20.0	27.0
	6.4	20.0	24.0

Note: Soil analysis was performed by the Agricultural Diagnostics Services of the College of Tropical Agriculture and Human Resources, University of Hawaii. Aluminum was extracted from soil samples with 1 mol·L⁻¹·KCl, and manganese was extracted with Mehlich III solution. The concentration of aluminum and manganese in the extracts were quantified by inductively coupled plasma emission spectrometry, and the instrument employed was a DV8000 emission spectrometer (Perkin-Elmer, Covina, California).

face™ at 10 g inoculum·70 g medium⁻¹ and transferring the mixture into dibble tubes. *Glomus aggregatum* was used in the study because it is a local isolate and has proven to be effective in enhancing the P uptake and growth of a wide array of host species grown on a variety of Hawaiian soils. Crude inoculums of *G. aggregatum* were produced by growing corn in sterilized Mansand™ (Ameron Hawaii Concrete and Construction, Honolulu, Hawaii (crushed basalt) that was inoculated with a starter culture of the fungus (1% by mass) and supplemented weekly with an application of Hoagland's solution (Hoagland and Arnon 1950) containing 8 mg P·L⁻¹, at 1000 mL solution·10 kg medium⁻¹. Additional details are published by Habte and Osorio (2001). The crude inoculum contained 5.3 infective propagules·g⁻¹. The noninoculated controls received 10 g of the heat-sterilized inoculum material.

Germinated seeds were selected for uniformity of radicle length and planted in the dibble tubes. For this purpose, 2.54 cm deep depressions were made in the centre of the moistened medium contained in each dibble tube with a clean forceps, and one germinated seed was planted in each depression with the radicle pointing downward. The dibble tubes were placed on racks and incubated on greenhouse benches that were covered with brown paper until the emergence of cotyledon leaves. After emergence, the brown paper was removed and the racks were placed under an automatic overhead sprinkler irrigation system. Plants were watered three times a day for durations of 5 min at a time. Macronutrients were supplied to the seedlings by adding the controlled release fertilizer Osmocote™ (19-6-12, 3–4 month release, Brewer Environmental, Honolulu, Hawaii) (12 g·kg medium⁻¹) to the surface of the medium contained in each dibble tube. Micronutrients were supplied as Hoagland's micronutrient solution (see Habte and Osorio 2001), which was administered at the rate of 20 mL·dibble tube⁻¹ every 2 weeks. The nutrient supplements were added beginning 20 d after emergence. After 7 weeks of growth, seedlings were removed from the dibble tubes, washed free of medium,

and transplanted into the potted soils described above after raising the moisture content of the soil to maximum water-holding capacity. One seedling was transplanted per soil-filled pot. Macronutrients were added to the soils at 12 g Osmocote-kg⁻¹ soil, which was spread over the surface of the soil. Micronutrients were added as Hoagland's micronutrient solution at the rate of 600 mL.pot⁻¹ every 2 weeks. Plants were grown under natural light in the greenhouse of the Department of Tropical Plant and Soil Science, University of Hawaii (21°51'N; 157°22'W). Plants were watered from saucers in which each pot rested so that water moved upward to the root zone by capillary action through perforations made at the bottoms and sides of the pots.

Experimental design

The experiment consisted of a factorial combination of two cultivars, two levels of mycorrhizal inoculation, and three levels of pH. Treatments were arranged on greenhouse benches in a completely randomized block design with three replicates per treatment.

Measurements

AMF colonization of roots

Roots were excised and carefully washed free of soil with water under pressure. The degree to which they were colonized by AMF was determined on a 0.5 g fresh mass root sample. The root samples were immersed into a 10% KOH solution for 24 h at 22 °C to clear roots. At the end of the incubation period, roots were rinsed with four changes of tap water. They were then acidified by soaking them in 10% HCl for 5–10 min to facilitate the retention of the staining material. After removing the excess acid, roots were stained by soaking them in a 0.15% acid fuchsin (dissolved in acidified glycerine). After 24 h of incubation at 22 °C, the stain was removed and the roots were then soaked in acidified glycerine and incubated at 22 °C for 24 h to destain them. The spent destaining solution was then replaced with a fresh destaining solution, at which time the roots were ready for observation. The stained roots were spread in Petri plates marked with gridlines and observed under a stereoscopic microscope, and the extent to which the roots were colonized was estimated by employing the gridline intersect method (Giovannetti and Mosse 1980).

Pinnule P content

The development of AMF symbiotic effectiveness was monitored by measuring pinnule P content of *Leucaena* leaves periodically, as described by Habte (1992). For this purpose, the third pinnule from the base of the pinna of the youngest fully expanded leaf of *Leucaena* was removed at convenient intervals. The pinnules were dried at 70 °C for 3 h and then transferred into 18 mm wide and 150 mm deep Pyrex test tubes. The contents of the test tubes were ashed in a muffle furnace at 500 °C for 3 h (Habte and Osorio 2001). The P content of the ash was determined spectrophotometrically after color was developed by the molybdenum blue technique (Murphy and Riley 1962). Appropriate calculations were made to express P content in micrograms per pinnule. Effectiveness of AMF expressed in this way and as P concentration of pinnules was previously determined to be remark-

ably similar (Habte 1994). Using the former approach eliminates the extra labor associated with pinnule mass determination.

Shoot biomass

After 14 weeks of growth, shoots were cut off above the soil line, and shoot dry-matter yield was determined after oven drying the samples to a constant mass at 70 °C.

Analyses of data

Data on total P accumulated in pinnules were obtained by separately integrating the areas under pinnule P content curves of the various treatments. These and other data were log transformed before they were subjected to analysis of variance (general linear model) using the statistical software Statistix for Windows, Version 8 (Analytical Software, Tallahassee, Florida). The least significant distance ($p < 0.05$) was used to separate treatment means when the F statistic was significant. Data from the two soils used were analyzed separately.

Results

Al-rich Leilehua soil

AMF colonization

AMF colonization of roots of *L. leucocephala* was significantly higher when seedlings of the legume were colonized by *G. aggregatum* before transplanting (inoculated) than when they were mycorrhiza free (noninoculated) (Fig. 1). Colonization was lowest for each cultivar at pH 4.5, with the exception of the colonization level of roots of cv. K-8 plants that were started from mycorrhizal seedlings, which was not different from the colonization level observed at pH 5.0. AMF colonization of cv. K636 and cv. K-8 started as mycorrhiza-free, and mycorrhizal seedlings responded significantly to one or both increments of pH (Fig. 1). However, mycorrhizal colonization levels observed on roots of cv. K-636 were generally higher than those observed on roots of cv. K-8 when plants were started from mycorrhiza-free seedlings. When plants were started from mycorrhizal seedlings, cultivar differences were pH dependent.

The extent of AMF colonization of roots of cv. K-636 and cv. K-8 at pH 4.5 was 78% and 46% of that observed at pH 6.2, respectively, when the plants were started from mycorrhiza-free seedlings (Table 2). When the plants were started from mycorrhizal seedlings, the levels of colonization observed in roots of cv. K-636 and cv. K-8 at pH 4.5 were 70% and 81.6% of that observed at pH 6.2, respectively. Arbuscular mycorrhizal inoculation, pH, and variety accounted for 69%, 17%, and 2% of the variance, respectively. Two- and three-way interactions were significant ($p < 0.05$), but altogether amounted to less than 10% of the variability.

Pinnule P content

Pinnule P content of noninoculated seedlings of both cultivars changed extremely slowly or did not change at all during the first 65 days after transplanting (Fig. 2). The values then increased sharply to approximately 2.5–4 μg P-pinnule⁻¹ by day 80, after which time they declined. At pH 4.5, pinnule P contents of inoculated and noninoculated cv. K-636 seedlings were similar for all sampling times except at day 20. Inocula-

Fig. 1. Effects of pretransplant inoculation of seedlings of two cultivars of *Leucaena leucocephala* with *Glomus aggregatum* and post-transplant bulk soil pH on the proportion of root length colonized by AMF 14 weeks after being transplanted into Al-rich Leilehua soil. Error bars represent least significant difference ($p = 0.05$).

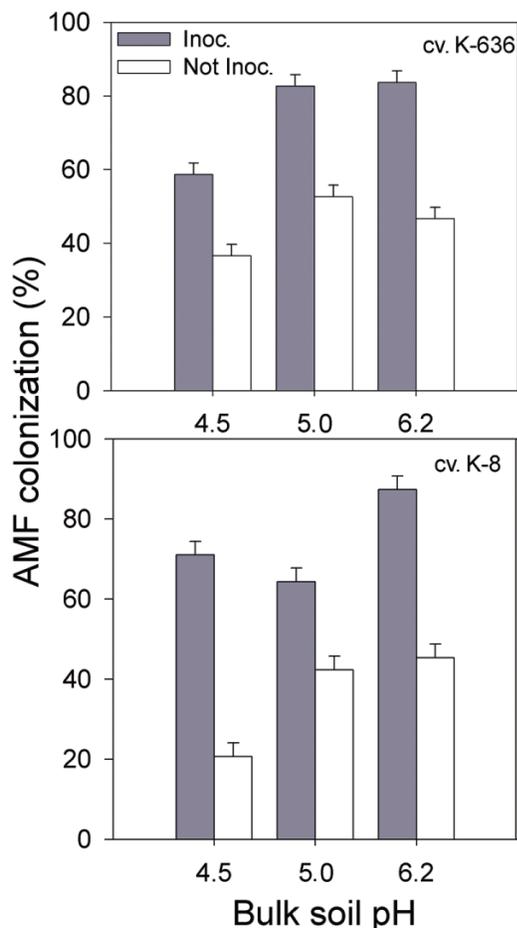


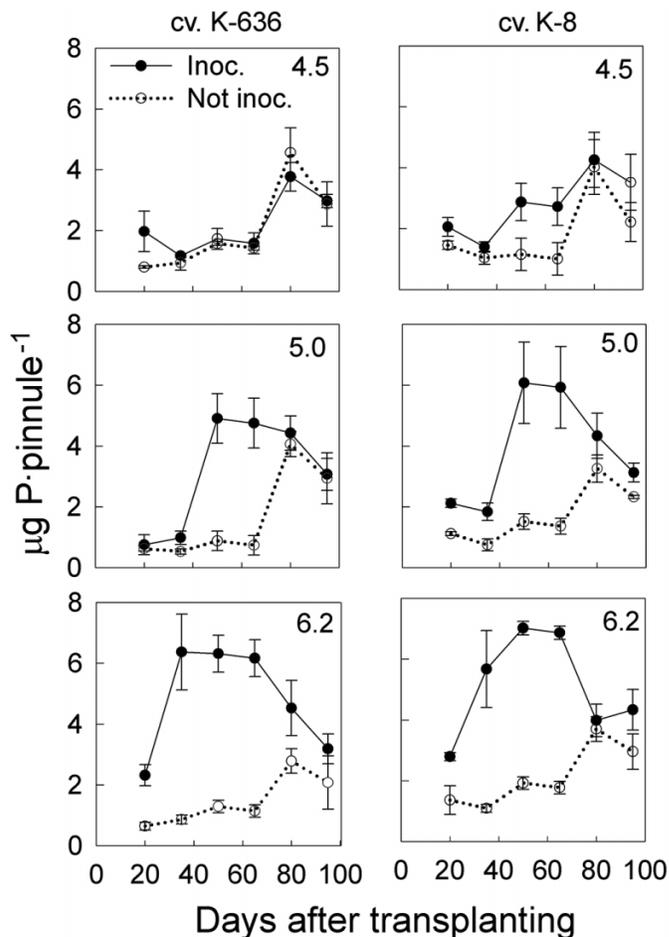
Table 2. Shoot biomass at pH 4.5 expressed as percent of that observed at the highest pH* of a soil.

Cultivar	Soil	pH	Yield (%)	
			Inoculated with AMF	Not inoculated
K-8	Leilehua	4.5	32.5	57.0
		5.0	51.9	94.0
	Wahiawa	4.5	32.5	50.0
		5.0	84.6	68.0
K-636	Leilehua	4.5	22.6	67.8
		5.0	41.6	83.0
	Wahiawa	4.5	35.0	40.3
		5.0	69.8	70.8

*6.2 for the Leilehua soil and 6.4 for the Wahiawa soil.

tion lead to significant increases in pinnule P content of cv. K-8 at days 50 and 65. The lag in pinnule P content of inoculated seedlings was limited to the first 2 sampling days at pH 4.5 and 5.0, whereas no lag was observed at pH 6.2. At pH 5.0, pinnule P content of inoculated cv. K-636 plants was higher than that of noninoculated cv. K-636 plants at days 50 and 65, whereas in cv. K-8, the values were higher at all

Fig. 2. Effects of pretransplant inoculation with *Glomus aggregatum*, post-transplant bulk soil pH, and cultivars of *Leucaena leucocephala* seedlings on pinnule P content of pinnules during the 14 week period after seedlings were transplanted into Al-rich Leilehua soil. Error bars represent SE.

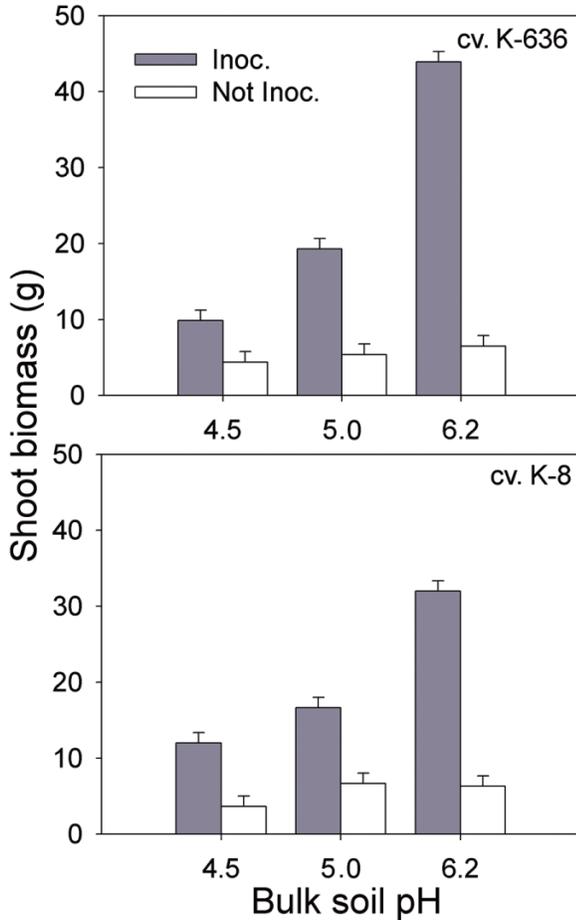


sampling days, except day 80. At pH 6.2, pinnule P content of inoculated plants was higher than that of noninoculated ones at all sampling days, except at day 95 for cv. K-636 and days 80 and 95 for cv. K-8. Total P content of pinnules calculated by integrating the areas under the pinnule P content curves increased with pH progressively in cv. K-636. In cv. K-8, total pinnule P content increased significantly only if pH had increased from 4.5 to 6.2. Total pinnule P content increased significantly by inoculation in both cultivars. Cultivar differences were not significant ($p < 0.05$). AMF inoculation and pH accounted for 75% and less than 5% of the variance, respectively. Two-way interaction between AMF inoculation and pH and between AMF inoculation and variety was significant. Three way interaction among AMF inoculation, variety, and pH was also significant and together accounted for 15% of the variance.

Shoot biomass

Shoot biomass of noninoculated cv. K-8 seedlings responded significantly to an increase in pH from 4.5 to 5.0 (Fig. 3). Such a response was not noted for cv. K-636. Liming the soil to pH 6.2 stimulated yield significantly in both cultivars. Increasing pH from 5.0 to 6.2 had no effect on the

Fig. 3. Average aboveground biomass of *Leucaena leucocephala* seedlings 14 weeks after being transplanted into Al-rich Leilehua soil as a function of pretransplant inoculation with *Glomus aggregatum*, post-transplant bulk soil pH, and cultivar. Error bars represent least significant difference ($p = 0.05$).



yield of these plants. In contrast, shoot biomass of inoculated seedlings progressively increased with increase in soil pH, and their biomass was significantly greater ($p < 0.05$) than the biomass of noninoculated plants at all levels of pH. Dry-matter yield obtained by inoculated seedlings at pH 4.5 was approximately 10–12 g·plant⁻¹ compared with 32–44 g·plant⁻¹, which was observed at the highest pH. AMF inoculation and pH accounted for 67% and 17% of the variance, respectively (Table 3). Variety had no significant influence on shoot biomass.

Mn-rich Wahiawa soil

AMF colonization

The levels of AMF colonization of roots of cv. K-636 and cv. K-8 generally increased with an increase in pH, regardless of the mycorrhizal status of the seedlings from which they were started (Fig. 4). However, the levels of colonization observed in this soil was much lower than that observed in the Leilehua soil. The levels of AMF colonization of roots observed was also appreciably higher for cv. K-636 than for cv. K-8. At pH 4.5, AMF colonization of roots of cv. K-636 and cv. K-8 plants that were started from mycorrhiza-free seed-

Table 3. Proportion of the total variance accounted for by treatment and interaction effects (%).

Effect	Measured variables		
	AMF colonization	Pinnule P	Shoot mass (g)
Wahiawa soil			
AMF	34.0	78.0	33.0
pH	37.0	6.2	37.8
Variety	14.0	ns	17.0
AMF × pH	ns	4.7	7.2
pH × variety	5.9	3.7	ns
AMF × variety	ns	ns	ns
AMF × variety × pH	ns	ns	ns
Leilehua soil			
AMF	69.0	75.0	67.0
pH	16.9	4.6	14.0
Variety	2.0	ns	ns
AMF × pH	1.8	8.4	ns
pH × variety	2.4	ns	ns
AMF × variety	1.3	2.0	ns
AMF × variety × pH	3.5	3.6	ns

Note: ns, not significant at $p < 0.05$.

lings were 37% and 46% of that observed at pH 6.4, respectively. When the plants were started from mycorrhizal seedlings, the respective values were 36% and 33%. AMF inoculation, pH, and variety accounted for 34%, 37%, and 14% of the variance in AMF colonization, respectively. Interaction between AMF and pH was significant ($p < 0.05$) and accounted for 5.9% of the variability.

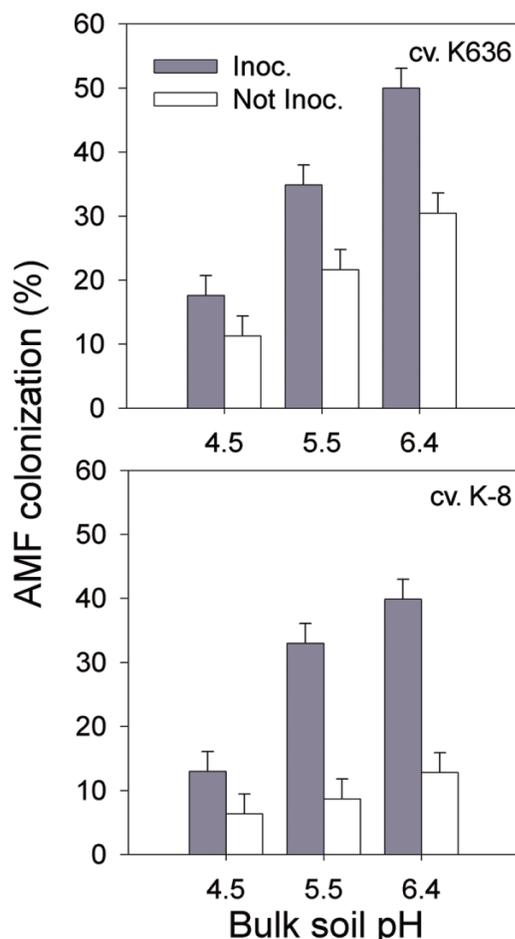
Pinnule P content

Pinnule P content of noninoculated seedlings changed slowly or not at all prior to day 65 (Fig. 5). It then increased, reaching peak values of 4–4.5 $\mu\text{g}\cdot\text{pinnule}^{-1}$ on day 80. Thereafter, the values declined. A different pattern was observed for most inoculated seedlings with pinnule P content increasing rapidly to peak values $>6 \mu\text{g}\cdot\text{pinnule}^{-1}$ on day 50. Cultivar cv. K-636 grown at pH 4.5 was the exception in that pinnule P content did not peak until day 80 and its value was $<6 \mu\text{g}\cdot\text{pinnule}^{-1}$. Analysis of data obtained by integrating the areas under the pinnule P content curves showed that total P content of pinnules was significantly affected by pH but not by cultivar. Total pinnule P content of noninoculated plants was stimulated significantly ($p < 0.05$) by increases in pH. pH had no effect on inoculated cv. K636 plants. Only the first increment in pH was associated with increase in total pinnule P content of cv. K-8. Plants inoculated with *G. aggregatum* had significantly ($p < 0.05$) higher total pinnule P content than that of noninoculated ones at all the pH levels tested. AMF inoculation and pH accounted for 78% and 6.2% of the variability in pinnule P content, respectively, and cultivar had no significant influence on the variable. Two-way interaction between AMF inoculation and pH and between pH and variety were significant and together accounted for 8.4% of the variance.

Shoot biomass

Shoot biomass increased with increase in pH (Fig. 6). The effect of AMF inoculation was significant for both cultivars

Fig. 4. Effects of pretransplant inoculation of seedlings of two cultivars of *Leucaena leucocephala* with *Glomus aggregatum* and post-transplant bulk soil pH on the proportion of root length colonized by AMF 14 weeks after being transplanted into Mn-rich Wahiawa soil. Error bars represent least significant difference ($p = 0.05$).

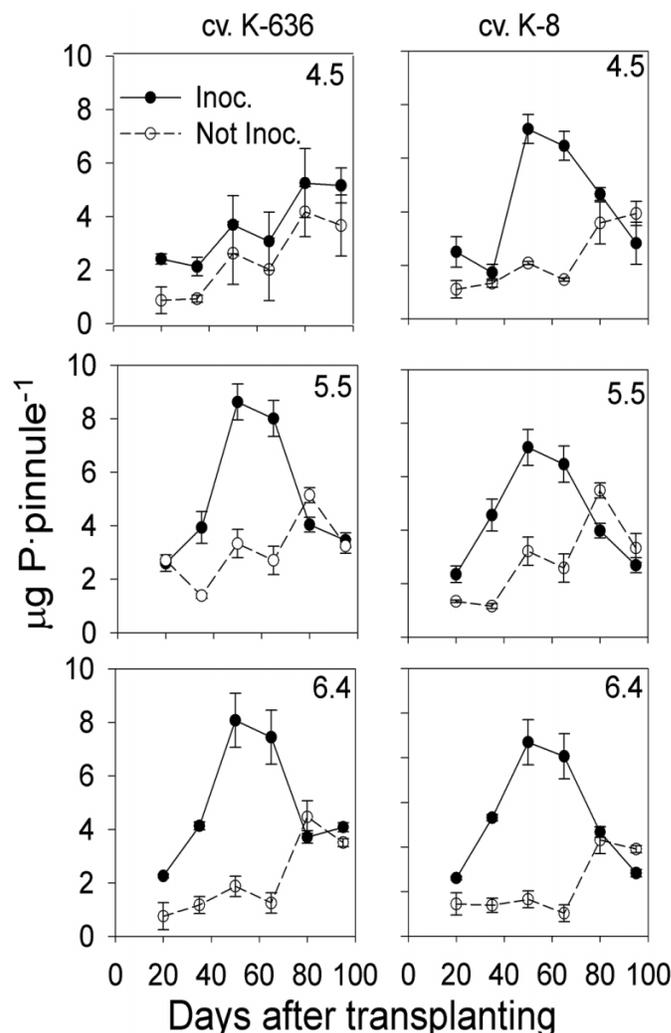


at all the pH levels tested. Shoot biomass of noninoculated cv. K-8 seedlings was smaller than that of noninoculated cv. K-636 seedlings at all pH values. Shoot biomass of inoculated cv. K-636 was also greater than that of inoculated cv. K-8 at pH 4.5 and 6.4, yields of the two cultivars being similar at the intermediate pH. Shoot biomass observed at pH 4.5 for cv. K-636 and cv. K-8 was 40% and 50% of that observed at pH 6.4, respectively, when plants were started from mycorrhiza-free seedlings. When the plants were started from mycorrhizal seedlings, the corresponding values were 35% and 33%, respectively. AMF inoculation, pH, and variety accounted for 33%, 37%, and 17% of the variability in shoot biomass, respectively (Table 3).

Discussion

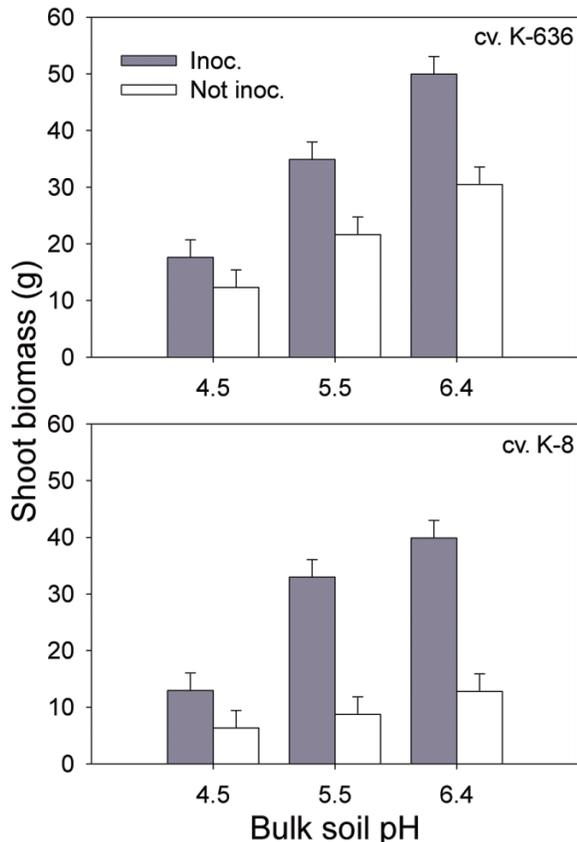
The infectivity and symbiotic efficacy of AMF were adversely affected by low pH in both test soils, and part of this adverse effect was offset by inoculating seedlings with *G. aggregatum* prior to transplanting them into the soils. The endophyte–host association was much more sensitive to acid soil toxicity in the Wahiawa soil than in the Leilehua soil. This difference appears to be a function of the higher content

Fig. 5. Effects of pretransplant inoculation with *Glomus aggregatum*, post-transplant bulk soil pH, and cultivars of *Leucaena leucocephala* seedlings on pinnule P content of pinnules during the 14 week period after seedlings were transplanted into Mn-rich Wahiawa soil. Error bars represent SE.



of Mn^{2+} in the former soil (Table 1). Both AM fungal colonization data and data on shoot biomass indicate that pH and AMF inoculation had comparable influence on these response variables in this soil, whereas pH effect was a relatively small component of the variance in the Leilehua soil. In the Mn-rich Wahiawa soil at pH 4.5, AMF colonization of roots of *L. leucocephala* was less profuse than in the Al-rich Leilehua soil, which suggested that both the hosts and the fungi were relatively more sensitive to acid soil toxicity in the Wahiawa soil. Although this soil is considered a Mn-rich soil, it also contained a large quantity of extractable Al^{3+} (Table 1). Therefore, plants grown in the Wahiawa soil at pH 4.5 were exposed to toxic levels of both Al^{3+} and Mn^{2+} . Evidently, the fungi were more sensitive to Mn^{2+} than to Al^{3+} . In the Leilehua soil, pretransplant inoculation of seedlings increased AMF colonization to reasonably high levels (57%–64%), even at pH 4.5, suggesting that the fungus had a relatively high degree of tolerance to Al^{3+} . The effect of inoculation on AMF colonization in the Wahiawa soil was

Fig. 6. Average aboveground biomass of *Leucaena leucocephala* seedlings 14 weeks after being transplanted into Mn-rich Wahiawa soil as a function of pretransplant inoculation with *Glomus aggregatum*, post-transplant bulk soil pH, and cultivar. Error bars represent least significant difference ($p = 0.05$).



quite restricted, apparently by the toxic effect of Mn^{2+} and (or) perhaps by the simultaneous presence of Al^{3+} and Mn^{2+} in the soil.

Data on AMF colonization (Figs. 1 and 4), pinnule P content (Figs. 2 and 5), and shoot biomass (Figs. 3 and 6) indicate that the infectiveness and effectiveness of the indigenous fungi was inferior to that of *G. aggregatum* and, hence, significant mycorrhizal inoculation effects were observed for all of these parameters across the pH ranges tested. This inferiority of the indigenous AMF can largely be explained by the low initial density of their infective propagules, as can be inferred from the long lag phase that we noted before symbiotic effectiveness measured as pinnule P content was expressed by the fungi (Figs. 2 and 5; Habte and Fox 1989). It is also conceivable that the growth rate of the indigenous AMF was inherently slow. However, when shoot biomass observed at pH 4.5 was expressed as a percent of that observed at pH 6.4, the values were consistently lower for plants that were started from mycorrhizal seedlings than those that were started from mycorrhiza-free seedlings (Table 2). Since the latter seedlings became colonized by indigenous AMF subsequent to transplanting, the values suggest that indigenous AMF were relatively more tolerant to acid soil toxicity compared with *G. Aggregatum* (Table 2). Isolating and multiplying these indigenous AMF for use in combination with acid

tolerant *L. leucocephala* cultivars should be part of the strategy for enhancing the establishment of the legume in acid soils.

The relative tolerance to acid soil toxicity of the cultivars tested was influenced by soil type and mycorrhizal status of the seedlings from which the plants were started (Table 2). In the Leilehua soil, cultivar had minimal effect on AMF colonization and had no effect on P uptake or shoot biomass. In the Wahiawa soil, cv. K-8 was relatively more tolerant to acid soil toxicity than cv. K-636 when plants were started from mycorrhiza-free seedlings, the difference disappearing when plants were started from mycorrhizal seedlings.

The decline in pinnule P content values after peak values had been reached is due to the translocation of more of the P from leaves to twigs and stems as the plants grew older (Habte 1992).

Most of the published work concerned with the role AMF play in the alleviation of acid soil toxicity deals with agronomic and pastoral species. The total number of articles focused on tree species is extremely limited, and they deal exclusively with three species, namely *Clusia multiflora* H.B.K. (Cuenca et al. 2001), *Liquidambar styraciflua* L. (Davis et al. 1983), and *Liriodendron tulipifera* L. (Lux and Cumming 2001).

A number of researchers did not include pH ranges that enable one to compare host response in the presence and absence of toxic levels of Al^{3+} and Mn^{2+} . Among the papers in which there is sufficient data for making such comparisons (none of which involved tree species), the data showed, as our data did, that AMF inoculation offset only a fraction of the potential yield lost owing to acid soil toxicity (Davis et al. 1983; Mendoza and Borie 1998; Kelly et al. 2005). In some cases, AMF inoculation had no effect (Rufyikiri et al. 2000), while in rare instances, AMF inoculation offset all of the yield lost owing to acid soil toxicity (Medeiros et al. 1994; Cuenca et al. 2001; Lux and Cumming 2001).

The mechanisms by which AMF alleviate Al^{3+} and Mn^{2+} toxicity in colonized plants is not clear. The claim that AMF reduce Al^{3+} toxicity by reducing the concentration of Al^{3+} in roots and shoots has not been consistently demonstrated. The literature indicates that the effect of the fungi is varied, sometimes reducing it (Yang and Goulart 1997; Mendoza and Borie 1998; Cuenca et al. 2001; Kelly et al. 2005), at times enhancing it (Medeiros et al. 1994; Qing and Goulart 1997; Lux and Cumming 2001), and at other times not affecting it at all (Cuenca et al. 2001). There is some indication that the fungi can sequester Al^{3+} in their hyphae, hyphal coils, vesicles, and auxiliary cells (Yang and Goulart 1997; Cuenca et al. 2001). While this phenomenon might serve as a mechanism for reducing the quantity of Al^{3+} reaching the root, its significance remains to be established. There is greater consensus that AMF alleviate Al^{3+} toxicity by offsetting the interference that Al^{3+} has on the uptake of nutrients, such as P, Ca, Mg, Cu, Zn, Mn, K, and Si (Medeiros et al. 1994; Clark and Zeto 1996; Mendoza and Borie 1998; Clark et al. 1999; Ruyikiri et al. 2000; Lux and Cumming 2001).

The role of AMF in ameliorating Mn^{2+} toxicity is even less clear. There are reports that suggest that AMF can reduce the amount of Mn^{2+} entering roots by suppressing bacteria that can increase the availability of Mn in the rhizosphere by solubilizing MnO_2 . However, this phenom-

enon has been demonstrated only at pH 5.7 and higher (Arines et al. 1989; Bethlenfalvay and Franson 1989; Kothari et al. 1991). Reports of work undertaken at lower pHs suggest that colonization by AMF generally enhance the uptake of Mn^{2+} by host plants (Medeiros et al. 1994; Clark and Zeto 1996; Clark et al. 1999; Lux and Cumming 2001). More recently, Nogueira et al. (2007) reported that the effect that AMF have on Mn status of soybean plants was time dependent, elevating tissue Mn content during the earlier phase of growth and reducing it during the latter phases of the growth of the plant. They concluded that the lower Mn content observed in mycorrhizal plants relative to nonmycorrhizal plants during the latter stages of growth was not due to reduced availability of Mn in the root zone, because AMF were associated with an increased concentration of Mn^{2+} in the rhizosphere.

Reactive Al^{3+} is barely present at pH 5.0 or higher, whereas Mn^{2+} can be present in toxic quantities at pH below 6.0 (Hue et al. 2001; Kochian et al. 2004). In the soils used in this study and in many acid soils, Mn^{2+} toxicity becomes a serious problem at pH < 5.6 and Al^{3+} ceases to be a serious problem at pH 5.0 and above (Hue et al. 2001; Kochian et al. 2004; Havlin et al. 2005). Consequently, in the Leilehua soil, Al^{3+} was problematic at pH 4.5, whereas in the Wahiawa soil, both Al^{3+} and Mn^{2+} were problematic. However, at pH 5.0 and 5.5, only Mn^{2+} toxicity was responsible for the adverse effects observed in the Wahiawa soil on AM fungal activity and growth of *Leucaena*.

The importance of considering genotypic variations among AMF species as one of the strategies for reducing the adverse effect of acid soil toxicity on host plants is evident from our data and from the works of others (Clark et al. 1999; Cuenca et al. 2001; Kelly et al. 2005). Moreover, our data argue that the strategy should also consider variations in host genotypes.

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