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M. Soedarjo · M. Habte

Mycorrhizal and nonmycorrhizal host growth in response to changes in pH and P concentration in a manganiferous oxisol

Abstract *Glomus aggregatum* and *Leucaena leucocephala* were allowed to interact in a manganese-rich oxisol at pH 4.3–6.0 and at soil P concentrations considered optimal for mycorrhizal host growth and sufficient for nonmycorrhizal host growth. At 0.02 mg P l^{-1}, vesicular-arbuscular mycorrhizal fungal (VAMF) colonization of roots increased as soil pH increased from 4.3 to 5.0. However, VAMF colonization of roots did not respond to further increases in pH. At pH 6.0, growth of mycorrhizal *Leucaena* observed at 0.02 mg P was comparable with that observed at 0.8 mg P l^{-1}. Increasing P concentration from 0.02 to 0.8 mg P l^{-1} increased target soil pH from 4.3 to 4.7 and reduced the concentration of available soil Mn from 15.1 to 1.9 mg l^{-1}. Thus, the normal plant growth observed at the higher P concentration at pH < 5 was mainly due to the alleviation of Mn toxicity as a result of its precipitation by excess P. VAMF colonization levels observed at pH 5.0–6.0 were similar, but maximal plant growth occurred at pH 6.0, suggesting that the optimal pH for mycorrhizal formation was substantially lower than for VAMF effectiveness. The poor growth of *Leucaena* at the lower P concentration in the unlimed soil was largely due to high concentrations of Mn^{2+} and H^+ ions.

Key words *Glomus aggregatum* · Hydrogen ion · *Leucaena leucocephala* · Manganese toxicity · Soil acidity · Target P concentration · Vesicular-arbuscular · VAMF colonization · VAMF effectiveness

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M. Soedarjo · M. Habte (✉)
Department of Agronomy and Soil Science,
University of Hawaii, 1910 East-West Road,
Honolulu, HI 96822, USA
Fax: (808) 956-6539

Introduction

Vesicular-arbuscular mycorrhizal fungi (VAMF) are increasingly being recognized for their ability to improve plant productivity in soils of low fertility. They do so largely by enhancing P uptake of associated plants (Bolan 1991). In many tropical soils in which plants are generally poorly supplied with P, VAMF could play an important role in improving plant productivity. A major obstacle to the realization of this potential, however, is the fact that these soils are acid (pH < 5.0) and that the impacts of soil acidity and associated toxicities on the formation and function of the vesicular-arbuscular mycorrhizal (VAM) symbiosis are not well understood (Foy 1983; Wang et al. 1985; Habte 1995). For example, VAMF improved plant growth after acid soils were limed in some instances (Davis et al. 1983), while in other acid soils significant mycorrhizal inoculation effects have been observed without liming (Guzman-Plazola et al. 1988). Whether these variations in response are due to variations in host species, endophyte species or both is often hard to tell. The aims of the current investigation were: (a) to determine the impacts of soil acidity on the development of the VAM symbiosis in a manganese-rich soil, and (b) to differentiate the impact of soil acidity on the host from its impact on the endophyte by interacting the two organisms at target soil solution P concentrations optimal for VAMF activity and sufficient for nonmycorrhizal host growth.

Materials and methods

Soil preparation

The soil used in this study was a moderately weathered oxisol (Wahiawa series, Rhodic Eutrustox, Clayey, Kaolinitic, Isohyperthermic). It was collected from a depth of 7.5–15 cm at the Poamoho Experimental Farm, University of Hawaii, Oahu, Honolulu. The soil had an initial pH of 4.9 and a high concentration of total Mn (Fox and Whitney 1981). Unless specified otherwise, all pH measurements were done using 1:2 soil/water suspension. Soil

Table 1 Chemical properties of Wahiawa soil before planting (*ND* not detected)

Target soil pH (1:2 H_2O)	Soil pH (1:2 0.01 M $CaCl_2$)	Nutrients in soil solution (mg l^{-1})			Nutrients in NH_4OAc extract (mg kg^{-1})	
		Mn	Mg	Ca	Mg	Ca
4.3	4.08	16.6	2.7	7.1	34	260
5.0	4.47	0.15	3.5	19.4	40	560
5.5	5.07	ND	3.7	40.4	36	1020
6.0	5.37	ND	3.9	63.8	24	1120

samples were air-dried and crushed to pass through a 4-mm sieve. Aliquots (2 kg) of the air-dried soil were transferred into 15′15-cm plastic pots for growing plants. In a preliminary study, 250-g (dry wt) portions of the soil were incubated with H_2SO_4 or $Ca(OH)_2$ at 28° C and at approximately 60% of water-holding capacity for 3 weeks in order to establish different target pH levels. Based on this incubation study, 0.025 M H_2SO_4 was required to obtain a pH of 4.3. The concentrations of $Ca(OH)_2$ needed to establish target pHs of 5.0, 5.5, and 6.0 were 0.0079, 0.024 and 0.0402 mol kg^{-1} soil, respectively. On the basis of the incubation study, $Ca(OH)_2$ or H_2SO_4 was added to 2-kg portions of the air-dry potted soil to attain the target pHs.

Two target soil solution P concentrations, namely 0.02 mg l^{-1} and 0.8 mg l^{-1} were established using the procedure described by Fox and Kamprath (1970). The former P concentration was found to be optimal for VAMF activity in *L. leucocephala*, while the latter concentration is known to be sufficient for nonmycorrhizal growth of the legume (Habte and Manjunath 1987) Phosphorus was added in the form of a KH_2PO_4 solution.

A crude inoculum of *Glomus aggregatum* Schenck and Smith emend Koske was used to inoculate the soils. The crude inoculum consisted of bits of infected roots, hyphae, spores and sand. Inoculation was achieved by thoroughly mixing 30 g of the crude inoculum with each 2 kg of dry soil. The inoculum was applied 1 day before planting.

Seeds of *L. leucocephala* var. K8 were scarified in concentrated H_2SO_4 (20 min) to break dormancy and to obtain seedlings free from pathogens. Seeds were then washed with sterilized water six times. Two seedlings were grown per pot, with thinning to one plant per pot 5 days later.

A blanket nutrient solution composed of KCl, $MgSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, H_3BO_3, $Na_2MoO_4 \cdot 2H_2O$, $ZnSO_4 \cdot 7H_2O$ and $Mg(NO_3)_2 \cdot 6H_2O$ was applied to obtain concentrations of 100, 50, 5, 10, 0.5, 10, and 69.2 mg of K, Mg, Cu, B, Mo, Zn, and N per kg of soil, respectively (Aziz and Habte 1987). Blanket nutrients were applied at planting.

The experiment was undertaken in the University of Hawaii Agronomy and Soil Science glasshouse under natural light (21° 51′ N, 156° 22′ W). Treatments were arranged in a randomized block design with three replicates per treatment. Pots were watered with deionized water as needed to maintain the soil at approximately water-holding capacity. Plants were grown for 45 days, from 13 June to 28 July 1991. Data were analyzed using the SAS procedure (SAS Institute 1991).

Measurements

Soil solution was extracted using the method of Adams et al. (1980) and was used to characterize selected chemical properties before planting and after harvest. The procedure developed by Habte et al. (1987) was employed to monitor the development of VAM activity. For this purpose, the third pinnule from the base of the youngest fully expanded leaf of *Leucaena* was sampled every 5 days beginning 15 days after planting. Pinnule samples were ashed for 3 h in a muffle furnace at 500° C before P was determined spectrophotometrically (Murphy and Riley 1962).

The degree of VAMF colonization of roots was assessed after roots were washed and stained using the method of Kormanik et al. (1980), except that we used 0.15% instead of 0.01% acid fuchsin. The stained roots were observed under a dissecting microscope and the degree of VAM colonization was assessed using the grid-line intersect method (Giovannetti and Mosse 1980). Above-ground plant parts were oven-dried at 70° C until constant weight was obtained and dry weight was recorded.

Oven-dried, above-ground plant parts were ground in a stainless steel Wiley mill. Portions (25 mg) of the ground tissue were ashed for about 4 h in a muffle furnace at 500° C. The ash was dissolved in 10 ml of 1 N HCl and digested to dryness on a hot plate. Afterwards, 10 ml of 1 N HNO_3 was added and the solution was analyzed for Ca, Mg, Mn, Cu and Zn by means of an atomic absorption spectrophotometer. Phosphorus was determined as described above.

Results

Soil chemical properties

The concentration of manganese in the soil solution measured before planting decreased with increase in soil pH and was negligible at pH 5.0. Soil solution magnesium and that extracted with ammonium acetate were not affected by soil pH. However, extractable Ca as well as Ca in the soil solution were significantly influenced by soil pH (Table 1).

After harvest, soil pH and Mn concentration in the soil solution remained relatively unchanged if the target P concentration was 0.02 mg l^{-1}, but increased from 4.3 to 4.7 if the target soil P concentration was elevated to 0.8 mg l^{-1} (Table 2). Concomitantly, soil solution Mn decreased from 15.1 mg l^{-1} to 1.9 mg l^{-1}.

Table 2 Soil chemical properties of Wahiawa soil after harvest (*ND* not determined). Values followed by the same letter under a given P level are not significantly different at the 5% probability level by the LSD test

Target soil pH	Soil pH (1:2 H_2O)	Soil Mn (mg l^{-1})
0.02 mg P l^{-1}		
4.3	4.36 d	15.1
5.0	4.98 c	ND
5.5	5.35 b	ND
6.0	5.67 a	ND
0.8 mg P l^{-1}		
4.3	4.76 d	1.9
5.0	5.31 c	ND
5.5	5.53 b	ND
6.0	5.88 a	ND

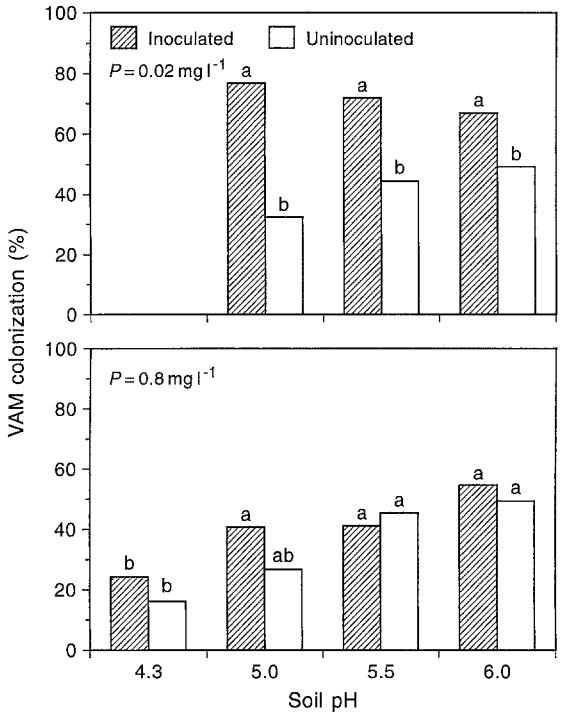

Fig. 1 The influence of VAMF inoculation, soil pH and P concentration on VAMF colonization of *Leucaena* roots. Histograms with a common letter are not significantly different at the 5% probability level by the LSD test

VAMF colonization of roots

At pH 4.3 and a target P concentration of 0.02 mg l^{-1}, roots of *Leucaena* were not colonized by VAMF; however, colonization was detected when the soil P concentration was increased to 0.8 mg P l^{-1} (Fig. 1), although the effect of inoculation on the variable was not significant. At target soil P concentration of 0.02 mg l^{-1}, inoculation of soil with *G. aggregatum* increased VAMF colonization of roots when soil pH was raised from 4.3 to 5.0. Further increases in pH did not influence colonization.

Development of VAM effectiveness

At pH of 4.3, plants grown at soil solution P concentration of 0.02 mg l^{-1} had lower pinnule P content than those grown at soil P concentration of 0.8 mg l^{-1}, but mycorrhizal inoculation did not significantly influence pinnule P content (Fig. 2a,b). At higher pH values, pinnule P content of plants grown in the inoculated and uninoculated soils became significantly different beginning at 20–30 days after planting.

Shoot and root dry weight

Shoot dry weight of *Leucaena* grown in soil with a target P concentration of 0.02 mg l^{-1} increased as soil pH was increased from 4.3 to 6.0. At this target P concentration, plant growth in inoculated and uninoculated soils was poor in the absence of lime. Above pH 4.3, plants in the inoculated soil grew better than those in the uninoculated soil (Fig. 3a). At a soil P concentration of 0.8 mg l^{-1}, maximal shoot dry weight of *Leucaena* was obtained in the inoculated soil at pH 6.0. However, response to mycorrhizal inoculation was greatest at target soil P concentration of 0.02 mg l^{-1}.

The effect of mycorrhizal inoculation on root dry weight was not evident at pH 4.3, irrespective of soil solution P status, although the growth of plants at the higher soil solution P concentration was significantly better (Fig. 3b). At higher pH values, VAMF inoculation stimulated root dry matter accumulation significantly at both soil solution P concentrations, greater stimulation being noted at 0.02 mg P l^{-1}.

Tissue nutrient status

Total shoot P content of *Leucaena* was significantly influenced by soil pH and mycorrhizal inoculation at both soil P concentrations tested (Fig. 4a). At a target soil P concentration of 0.02 mg l^{-1}, total shoot P content was higher when soil was inoculated with *G. aggregatum* than when it was not. At the higher soil P concentration, mycorrhizal inoculation increased shoot P content at pH 5.0 and 6.0 but not at pH 5.5.

Inoculation of soil with *G. aggregatum* increased shoot Cu content of *Leucaena* if soil pH was above 4.3 irrespective of soil P concentration (Fig. 4b). However, shoot Cu content of *Leucaena* observed at pH 6.0 was not significantly different from that observed at pH 5.5 at either soil P concentration.

Tissue shoot Zn content of *Leucaena* grown in the inoculated soil was higher than that grown in the uninoculated soil when pH was increased from 4.3 to 5.0 at both soil P concentrations. Further increases in soil pH did not increase shoot Zn content (Fig. 5a).

Shoot Mn concentration of *Leucaena* was mainly influenced by soil pH (Fig. 5b). The highest shoot Mn concentration was observed when *Leucaena* was grown at a target pH of 4.3 at either P concentration tested. Pronounced reduction of shoot Mn occurred when the soil pH was increased to 5.0 and 6.0.

At a soil P concentration of 0.02 mg l^{-1}, total shoot Ca content increased significantly in response to mycorrhizal inoculation when the soil pH was higher than 4.3, the highest shoot Ca content being observed in the inoculated soil at pH 6.0. (Fig. 6a). Total shoot Ca content did not respond to mycorrhizal inoculation at a target P concentration of 0.8 mg l^{-1}. It did, however, respond to increases in soil pH.

Shoot magnesium content was increased by liming and mycorrhizal inoculation at target P concentration of 0.02 mg l^{-1} but not at 0.8 mg l^{-1} (Fig. 6b). However, liming the inoculated soil to a pH higher than 5.0 did not result in a further increase in shoot Mg content.

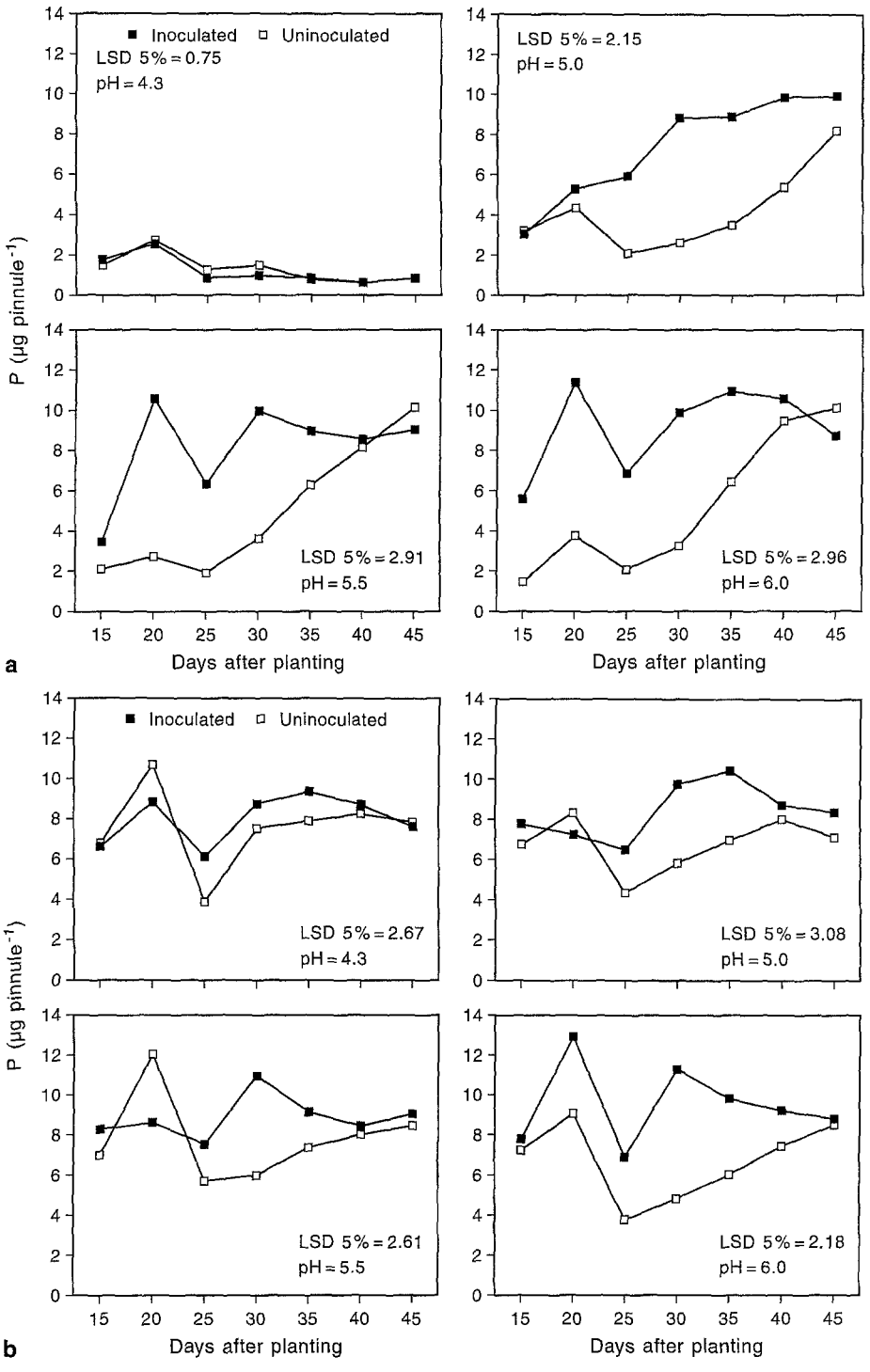

Fig. 2 The influence of VAMF inoculation and soil pH on the development of mycorrhizal effectiveness in *Leucaena* grown in soil at a target P concentration of **a** 0.02 mg P l^{-1} and **b** 0.8 mg P l^{-1}

Discussion

Changes in soil chemical properties

Our findings showed that the formation and effectiveness of the VAM symbiosis were significantly curtailed in the Wahiawa soil at pH 4.3 due to high concentrations of H^+ and Mn^{2+} in the soil solution. The adverse effect of these ions, particularly Mn, could be eliminated by increasing pH to 5.0. Our results agree with those of Fox and Whitney (1981) and Haynes and Swift (1985), who noted a significant decrease in extractable Mn at pHs higher than 5. Mn availability decreases with increase in soil pH because at high pH, Mn precipitates out of solution as $Mn(OH)_2$ (Ritchie 1989).

The decrease in Mn concentration and increase in pH in response to high P amendment may partly have to do with the fact that at pH 4.3 the Wahiawa soil is predominantly positively charged. Under this condition, excess $H_2PO_4^-$ ions due to high P fertilizer application could partly replace OH^- ions from adsorption

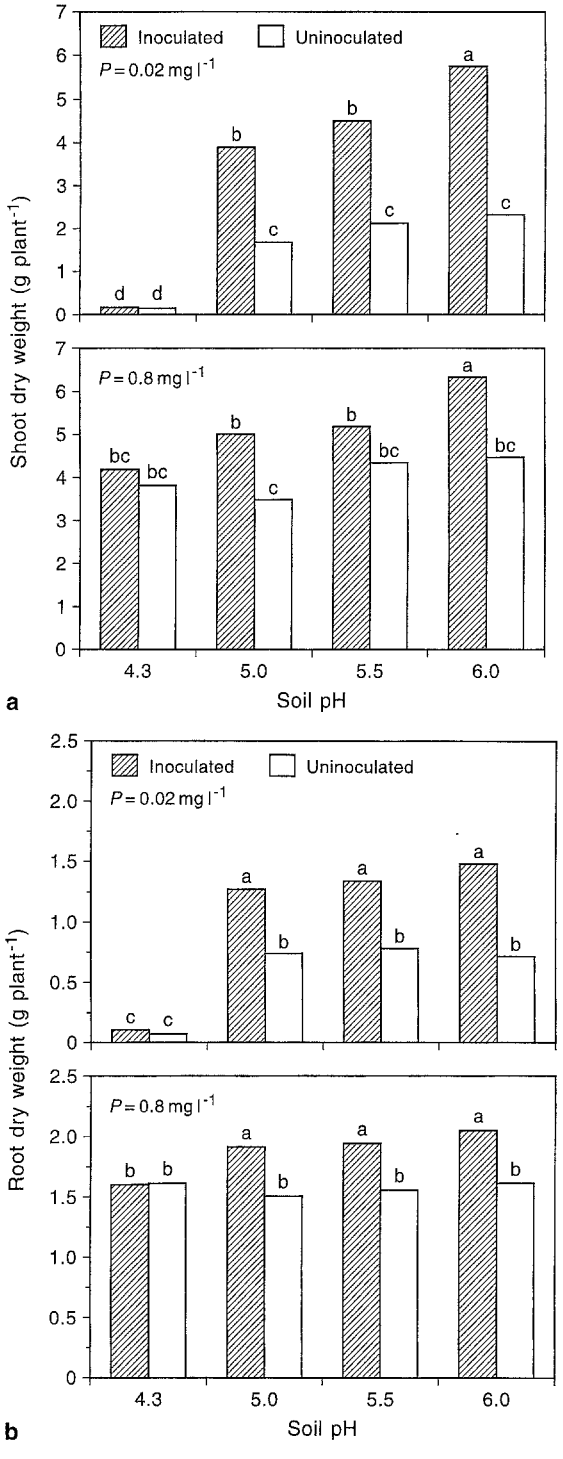

Fig. 3 The influence of VAMF inoculation, soil pH and P concentration on **a** shoot dry weight and **b** root dry weight of *Leucaena*. Histograms with a common letter are not significantly different at the 5% probability level by the LSD test

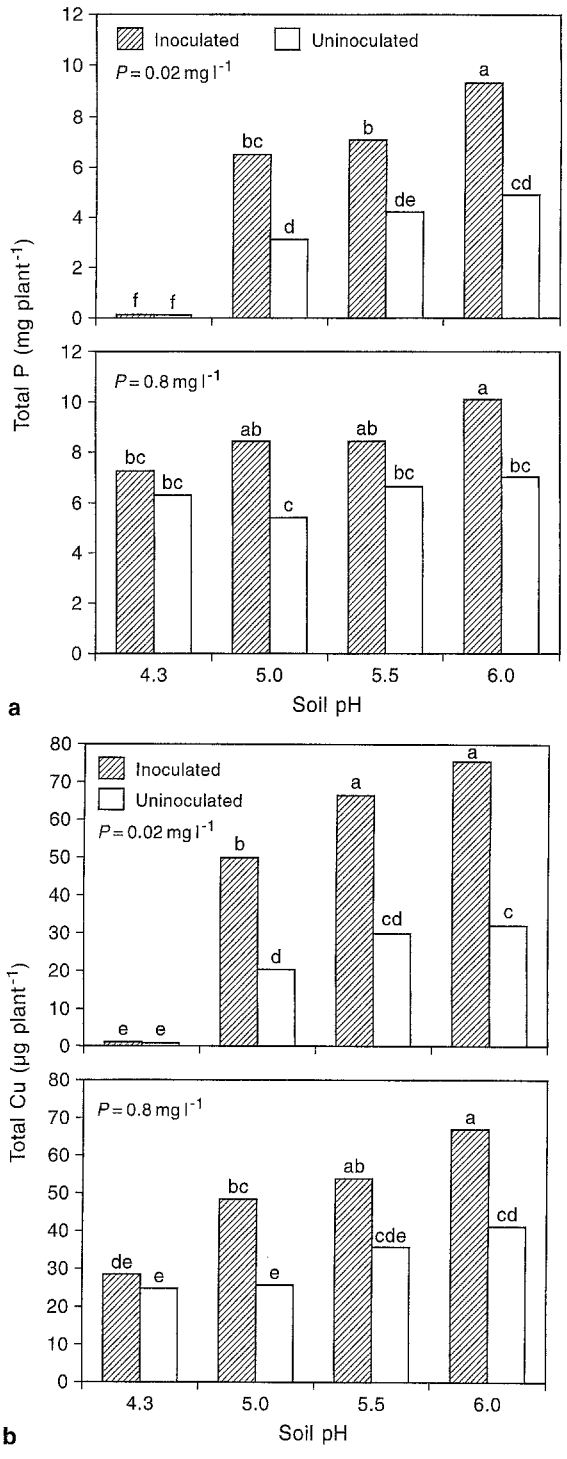

Fig. 4 The influence of VAMF inoculation, soil pH and P concentration on **a** total shoot P content and **b** total shoot Cu content of *Leucaena*. Histograms with a common letter are not significantly different at the 5% probability level by the LSD test

sites. The hydroxyl ions then go into the soil solution, thereby increasing soil pH (Uehara and Gillman 1981). Most of the reduction in Mn concentration, however, may be explained by the precipitation of divalent Mn from solution as $Mn_3(PO_4)_2$ in the presence of excess phosphate anions (Norvell 1988). It is perplexing that this decrease in soil solution Mn was not accompanied by a decrease in tissue Mn concentration (Fig 5b). The effect may have been obscured by the dilution and/or concentration effect of growth.

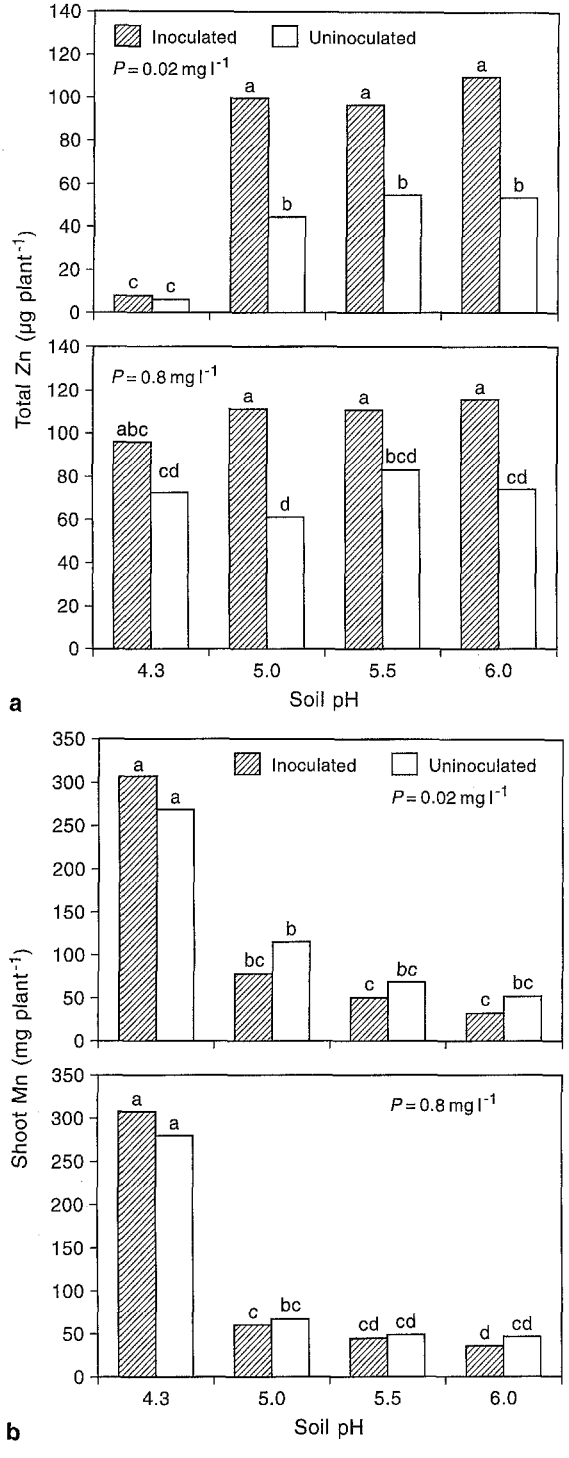

Fig. 5 The influence of VAMF inoculation, soil pH and P concentration on **a** total shoot Zn content and **b** total shoot MN content of *Leucaena*. Histograms with a common letter are not significantly different at the 5% probability level by the LSD test

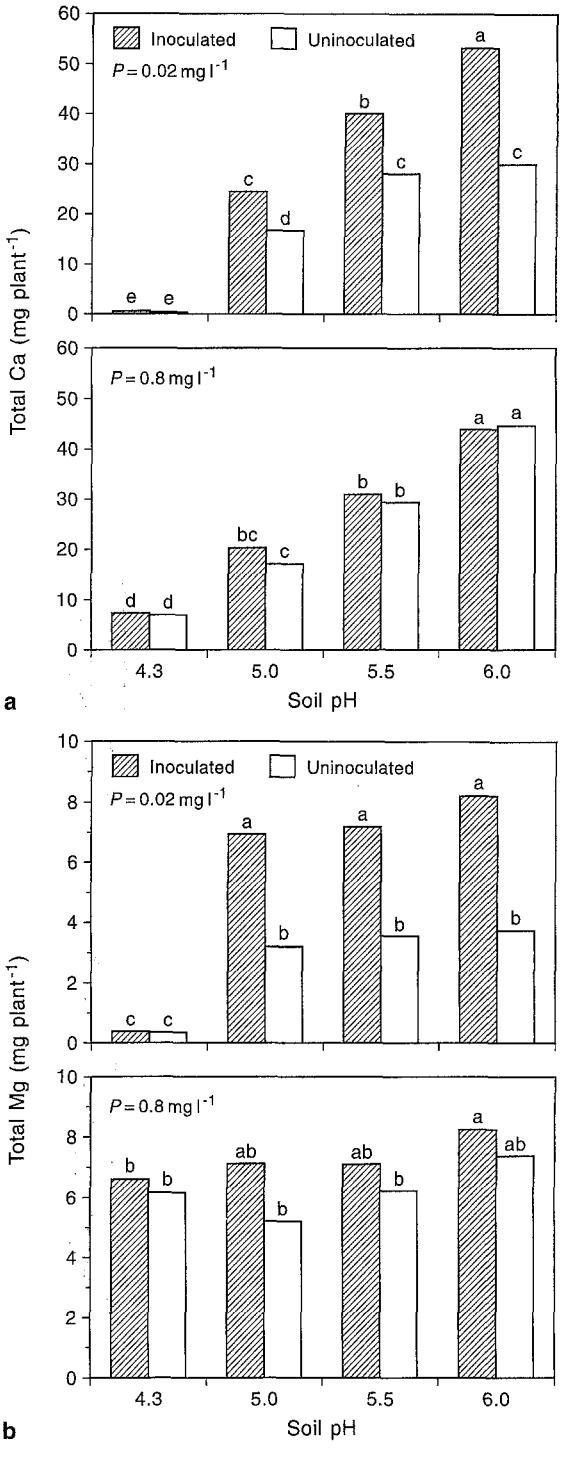

Fig. 6 The influence of VAMF inoculation, soil pH and P concentration on **a** total shoot Ca content and **b** total shoot Mg content of *Leucaena*. Histograms with a common letter are not significantly different at the 5% probability level by the LSD test

VAMF colonization of roots

Although VAMF colonization of *Leucaena* was severely limited in the unlimed soil, colonization of roots was insensitive to pH increases above 5.0. The low level of colonization we observed at the higher soil P concentration is consistent with the well-known fact that high P in roots depresses VAMF colonization (Koide and Mingguang 1990) by inducing deficiency of soluble carbohydrates (Jakobsen and Rosendahl 1990; Thompson et al. 1990).

At pH 5.0–6.0, the concentration of Mn was negligible (Tables 1, 2), and thus Mn is unlikely to adversely affect mycorrhizal formation at these pH levels. At a soil P concentration of 0.02 mg l^{-1}, the lack of mycorrhizal colonization in the unlimed soil may be related to high H^+, Mn^{2+}, low Ca^{2+} or to the combined effects of these cations. This observation is supported by the soil test data, which showed that the concentrations of H^+ and Mn^{2+} were high while that of Ca^{2+} was low (Tables 1, 2).

Development of VAMF effectiveness

As with VAMF colonization, the development of VAMF effectiveness indicated by pinnule P content was severely curtailed in the unlimed soil. The higher pinnule P content we observed at pH 6.0 compared to pH 5.5 or 5.0 was not due to greater root colonization, because VAMF colonization levels at these pH levels were similar (Figs. 1,2a). However, it may be related to the favorable effect of high Ca on VAMF effectiveness. The lack of relationship between mycorrhizal colonization and VAMF effectiveness could be due to the fact that VAMF colonization was determined at harvest (45 days after planting) while VAMF effectiveness was monitored throughout the study period. Manjunath and Habte (1988) found a positive correlation between VAMF colonization and mycorrhizal effectiveness in *Leucaena* during the first 35 days but not beyond this period. The observed lack of agreement could also be due to differences in the degree to which internal and extramatrical hyphae responded to changes in pH.

Shoot and root dry weight

The response of shoot dry weight of *Leucaena* to soil acidity was consistent with VAMF effectiveness determined as pinnule P content and shoot P content determined at harvest but not with VAMF colonization and root dry weight. The positive correlation we noted between VAMF effectiveness measured as pinnule P content and shoot P content and that between shoot P content and shoot dry weight is in agreement with the observations made by Habte et al. (1987).

The higher shoot dry weight of *Leucaena* in the inoculated soil at pH 6.0 compared to those observed at pH 5.0 and 5.5 was not related to higher root dry weight. It is probable that the quantity and quality of extramatrical hyphae produced at the highest pH were greater than that produced at lower pH.

Growth of *Leucaena* at a target soil P concentration of 0.02 mg l^{-1} was comparable with that observed at a target soil P level of 0.8 mg l^{-1} if the former soil was inoculated with *G. aggregatum* (Fig. 4). This represents a 40-fold decrease in the external P requirement of the legume due to inoculation with *G. aggregatum.*

The significant response to VAMF inoculation observed at a soil P concentration considered to be sufficient for the growth of nonmycorrhizal plants is partly explained by the very high dependency of *Leucaena* on VAMF for nutrient uptake and growth (Habte and Manjunath 1991). It is also possible that some of the P added to the test soil became inaccessible to the unaided *Leucaena* roots as time progressed.

At pH 4.3 and a P concentration of 0.02 mg l^{-1}, leaves of *Leucaena* exhibited marginal necrosis, a typical symptom of Mn toxicity. Consequently, plants were stunted. The cultivar of *Leucaena* used in the current study is reported to be sensitive to soil acidity (Halinda 1988). The lack of effect of mycorrhizal inoculation in the unlimed inoculated soil may be due to the toxic effects of high H^+ and/or Mn^{2+} prevailing at this pH. Wang et al. (1985) evaluated the effect of Mn on mycorrhizal formation in a sand culture and observed a fourfold decrease in fractional infection as the concentration of Mn increased from 0 to 5 mg l^{-1}. In the current study, VAMF activity may have also been restricted by inadequacy of Ca, since the concentration of Ca in the unlimed soil was low (see Tables 1, 2). The better plant growth observed at the higher than at the lower P concentration at this pH is explained by the lower Mn^{2+} content and to a lesser degree by the lower concentration of H^+.

The soil used in the current study was not sterilized; therefore, low levels of mycorrhizal colonization and effectiveness were observed in the uninoculated soil (Fig. 2a,b). The inferiority of the indigenous VAMF to that of *G. aggregatum* is explainable by the very low density of indigenous VAMF propagules in the soil, since the test soil was from a subsurface layer, i.e, the layer containing most of the indigenous VAMF propagules were removed before the soil was collected.

The Wahiawa soil was inherently low in Ca, and liming increased the concentration of Ca in the soil solution (see Table 1). The better response of *Leucaena* to VAMF inoculation irrespective of target P concentration after liming is thus partly due to the better supply of Ca. Soedarjo and Habte (1993) observed a similar kind of response after liming a Ca-deficient ultisol inoculated with *G. aggregatum.*

P is critical to root development (Salisbury and Ross 1985), and often VAMF-induced P uptake is accompanied by increases in root mass (Aziz and Habte 1987). Some of the results of the current investigation support these observations, while others do not. For instance, VAMF enhancement of P uptake did not lead to increase in root mass at pH 6.0.

Nutrient status of plant tissue

The effect of soil pH on total shoot P paralleled that of its effect on the development of mycorrhizal effectiveness, suggesting a close relationship between the earlier

development of VAMF effectiveness and total P uptake at harvest (Habte et al. 1987).

Although some of our results support the findings of other investigators regarding VAMF enhancement of Cu and Zn uptake (Lambert et al. 1979; Manjunath and Habte 1988), some of our data point to the fact that the effectiveness of mycorrhizal fungi in increasing the uptake of these micronutrients is significantly influenced by soil pH.

The similarity in shoot Mn content of *Leucaena* we observed irrespective of VAMF colonization indicates that VAMF did not play a significant role in Mn uptake. These results are in disagreement with the reports of Bethlenfalvay and Franson (1989) and Arines et al. (1989) in which it was suggested that mycorrhizal formation within roots of plants can reduce Mn uptake by associated plants.

Ca and Mg are mostly transported to the root zone by mass flow (Marschner 1986); consequently the higher Ca and Mg content in *Leucaena* in the inoculated soil, particularly at pH 6.0, is not a direct result of mycorrhizal formation. It may, however, be related to VAMF-mediated increases in tissue P content. Lambert et al. (1979) noted that mycorrhizal colonization did not directly increase uptake of Ca and Mg but that the ions may be required to balance negative charges resulting from the enhanced uptake of P due to mycorrhization. It may also reflect enhanced uptake of the nutrients as a result of mycorrhizal enhancement of root growth.

Conclusions

In order to maximize the benefits of the VAM symbiosis in acid soil containing large quantities of total manganese, it appears that soil pH should be increased sufficiently to alleviate Mn toxicity. However, higher pH levels may be required for maximal expression of VAMF effectiveness, at least in some species. The results of our study suggest that high concentrations of manganese and/or hydrogen ions and perhaps inadequacy of Ca could curtail the formation and effectiveness of VAMF in acid soils unless efforts are made to identify host-endophyte combinations that are tolerant to soil acidity and associated problems.

In view of the fact that P added to sustain nonmycorrhizal host growth influenced the concentrations of H^+ and Mn^{2+} in the soil solution, we were unable to completely separate the effects of soil acidity on VAMF endophytes from its effect on the plant.

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