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N. A. Onguene · M. Habte

Nitrogen and phosphorus requirements for raising mycorrhizal seedlings of *Leucaena leucocephala* in containers

Abstract A greenhouse study was undertaken to determine the nitrogen and phosphorus fertilization requirements for raising mycorrhizal seedlings in soil in containers. Seedlings of *Leucaena leucocephala* were grown for 40 days in dibble tubes containing fumigated or nonfumigated soil uninoculated or inoculated with *Glomus aggregatum*. The soil was fertilized with NH_4NO_3 solution to obtain 25–200 mg N kg^{-1} soil, and with a KH_2PO_4 solution to establish target soil solution P concentrations of 0.015–0.08 mg P l^{-1}. At the end of 40 days, seedlings were transplanted into pots containing 5-kg portions of fumigated soil. Posttransplant vesicular arbuscular mycorrhizal fungal (VAMF) effectiveness, measured as pinnule P content, plant height, shoot dry weight and tissue N and P concentrations, was significantly increased by pretransplant VAMF colonization in both soils. The best posttransplant mycorrhizal colonization and mycorrhizal growth responses were observed if the nonfumigated pretransplant soil was amended with 50 mg N kg^{-1} soil and 0.04 mg P l^{-1} or if the fumigated pretransplant soil was amended with 100 mg N kg^{-1} soil and 0.04 mg P l^{-1}. There was no relationship between N:P ratios of nutrients added to the pretransplant soil medium and shoot N:P ratios observed after transplanting. Shoot N:P ratio was also not correlated with root colonization level.

Key words *Glomus aggregatum* · Pinnule P · Posttransplanting · Pretransplanting · Shoot N · Shoot P · Shoot N:P ratio · VAMF colonization · VAMF effectiveness

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Introduction

Although pretransplant inoculation of container-grown seedlings with vesicular-arbuscular mycorrhizal fungi (VAMF) has been claimed to be one of the cost-effective ways to harness VAM endophytes in agriculture (Plenchette et al. 1983; Sreeramulu and Bagyaraj 1986), information on the management practices required for raising VAMF-colonized seedlings in containers, particularly using soils as pretransplant growth media, is non-existent.

Pretransplant VAMF colonization is likely to save time in seedling production (Vinayak and Bagyaraj 1990) as well as reduce fertilizer inputs during the pretransplant and posttransplant growth phases. Pretransplant colonization of seedlings with VAMF in containers may also enhance posttransplant survival and establishment of seedlings (Menge et al. 1978a; Cornet et al. 1982; Sasa et al. 1987) and may require less inoculum than direct field inoculation, as well as provide a competitive advantage to introduced VAMF over indigenous ones.

Seedlings raised in nurseries are generally supplied with large quantities of N and P to promote vigorous early growth. However, high tissue P or high concentrations of available soil P have been shown to decrease the proportion of root length colonized by VAMF (Mosse 1973; Menge et al. 1978b; Miranda et al. 1989). Though the effect of N is less well understood than that of P, high levels of soil N could have negative effects on VAMF development and VAMF-induced growth stimulation (Kruckelman 1975; Azcon et al. 1982; Hayman 1982). On the other hand, VAMF development and function could be hampered by an inadequacy of P or N in soil (Hepper 1983; Bolan et al. 1984; Hall et al. 1984; Habte and Manjunath 1987; Aziz and Habte 1990).

The purpose of the present investigation was to determine the appropriate combinations of N and P amendments required for raising VAMF-colonized see-

dlings in dibble tubes, based on the posttransplant growth responses of *Leucaena leucocephala*.

Materials and methods

Soil preparation and treatment

The soil used in this investigation was a subsurface sample (10–25 cm) of an ultisol (Clayey, Oxidic, Isothermic, Typic Kandihumult, Leilehua series) with an initial pH of 5.3 (1:2 soil-water suspension). A subsurface sample was chosen because of its low soil solution P concentration (0.015 mg l^{-1}), and to minimize the influence of organic P on P availability. After air-drying the soil on greenhouse benches for 5 days, it was crushed to pass through a sieve with a 2-mm pore size for raising seedlings or through a sieve with a 4-mm pore size for posttransplant growth of the seedlings. A lime requirement curve was used to adjust the pH of the soil to 6.0 using $Ca(OH)_2$ (1.66 g kg^{-1} soil) in order to minimize the effects of aluminum toxicity on root development.

Portions (0.5 kg dry weight basis) of the sieved (2-mm) soil were transferred into 4-l plastic pots. Nitrogen was added as NH_4NO_3 at the rate of 25, 50, 100, and 200 mg kg^{-1} soil. Other essential nutrients were supplied as prescribed by Habte and Manjunath (1987). The soil samples were then thoroughly mixed and incubated for 6 days under greenhouse conditions to achieve suitable moisture content (25%) for fumigation. Half of the pots containing the treated soil were fumigated with 48 g methyl bromide and 1.0 g chloropicrin in a gas-tight chamber for 4 days. After fumigation, the pots were removed from the chamber, placed on greenhouse benches and left to stand for 3 months to allow the fumigant to dissipate.

Six days before mycorrhizal inoculation, the soils were amended with KH_2PO_4 solutions to establish target soil solution P concentrations of 0.015, 0.02, 0.04 and 0.08 mg l^{-1} using the procedure developed by Fox and Kamprath (1970). After P addition, the soil was allowed to air-dry under greenhouse conditions for 3 days. The soil samples were then thoroughly mixed to enhance exchange and sorption reactions.

Mycorrhizal inoculation

One day before planting, soil in half of the pots was inoculated by mixing the contents of each pot with 15 g of crude inoculum of *Glomus aggregatum* Schenck and Smith emend Koske, consisting of sand, extramatrical spores and sporocarps, bits of hyphae and infected corn root segments. Uninoculated controls received 100 ml of a filtrate obtained by passing a suspension of the crude inoculum (50 g l^{-1}) through Whatman No. 1 filter paper three times.

Seedling growth medium, seed treatment and planting in dibble tubes

Portions (120 g) of the soils prepared above were transferred into yellow, UV-stabilized 3.81′20.9-cm dibble tubes (Stuewe and Sons, Corvallis, Ore.). Dibble tubes were provided with a 15-cm-long polyester wick (Wright, West-Waren, Mass.). The wick was allowed to extend from the dibble tubes into a reservoir containing deionized water to maintain the soil moist by capillary movement. Space not occupied by the wick at the bottom hole was plugged with styrofoam stoppers.

Seeds of *Leucaena leucocephala* K636 were pregerminated for 3 days in water agar (0.9%) at 30° C after scarification in concentrated H_2SO_4 for 20 min and rinsing with sterile water six times. One pregerminated seedling was planted per dibble tube. Seedlings were grown in dibble tubes for 40 days in the greenhouse under natural light (21° 51′ N, 56° 22′ W) from 30 August 30 to 9 October 1992.

Preparation of the post-transplant growth media

Following growth in dibble tubes, seedlings were transplanted in pots containing 5-kg portions (dry weight basis) of the Leilehua soil, after liming it to pH 6.0, and amending it with all essential nutrients (except P) (Habte and Manjunath 1987). The soil was fumigated as described above. Seven days before transplanting, a P solution was added to the soil to attain the target soil solution P concentration of 0.02 mg l^{-1}, a concentration determined to be optimal for mycorrhizal growth of *L. leucocephala* (Habte and Manjunath 1987).

Transplants were pulled out of dibble tubes, washed to remove soil particles, and placed in depressions made in the potted soils. Pots were arranged on greenhouse benches in a randomized complete block design with three replicates per treatment. Plants were grown in the greenhouse under natural light from 10 October to 26 November 1992. Pots were watered as needed to maintain soil moisture at approximately field capacity.

Measurements taken

The proportion of root colonized by VAMF was determined by the grid-line intersect method (Giovanetti and Mosse 1980) after clearing roots with 10% KOH and staining with acid fuchsin (0.15% in lactic acid-glycerol solution) (Kormanik et al. 1980).

The development of VAMF effectiveness was monitored by measuring the P content of pinnules of the youngest fully expanded leaf of *Leucaena* plants (Habte 1992) at 7-day intervals, starting on the 7th day after transplanting. Plant height was measured at transplanting, 21 days after transplanting and at harvest; it was measured from the cut collar to the tip of the stem where the newest terminal leaves were formed. Immediately after harvest, shoots were dried at 70° C for 48 h and weighed.

Phosphorus concentration in shoot samples was determined by the phosphomolybdate method (Murphy and Riley 1962) after dry-ashing 0.1-g portions of oven-dried and ground plant samples in a muffle furnace at 500° C for 4 h. Nitrogen contents of shoot samples were determined by digesting 0.25-g portions of shoot samples according to the Kjeldahl procedure (Chapman and Pratt 1961). The digests were then steam-distilled in the presence of 10 N NaOH. The distillate was collected in a 250-ml Erlenmeyer flask containing 40 ml of a 2% boric acid solution and methyl red-bromocresol indicator. The resulting solution was titrated with 0.05 N sulfuric acid.

Analysis of data was carried out using the SAS procedure (SAS Institute 1985). Simple linear correlation coefficients were estimated to establish relationships between shoot N:P ratios 47 days after transplanting (DAT) and N:P ratios of nutrients added to the pretransplant soil medium, and between shoot dry weight, root colonization, and shoot N:P ratios.

Results

VAMF colonization of roots after transplanting

Pretransplant inoculation of fumigated and nonfumigated soils with *Glomus aggregatum* increased VAMF colonization of roots significantly after transplanting; roots were colonized to the same extent in the two soils (Table 3, Fig. 1). Roots of *Leucaena* raised in the fumigated uninoculated pretransplant soil were not colonized by VAMF. On the other hand, VAMF colonization was observed in roots of plants raised in the nonfumigated uninoculated pretransplant soil, although the extent of colonization was low. Percent root colonization increased with increasing N levels up to 100 mg N

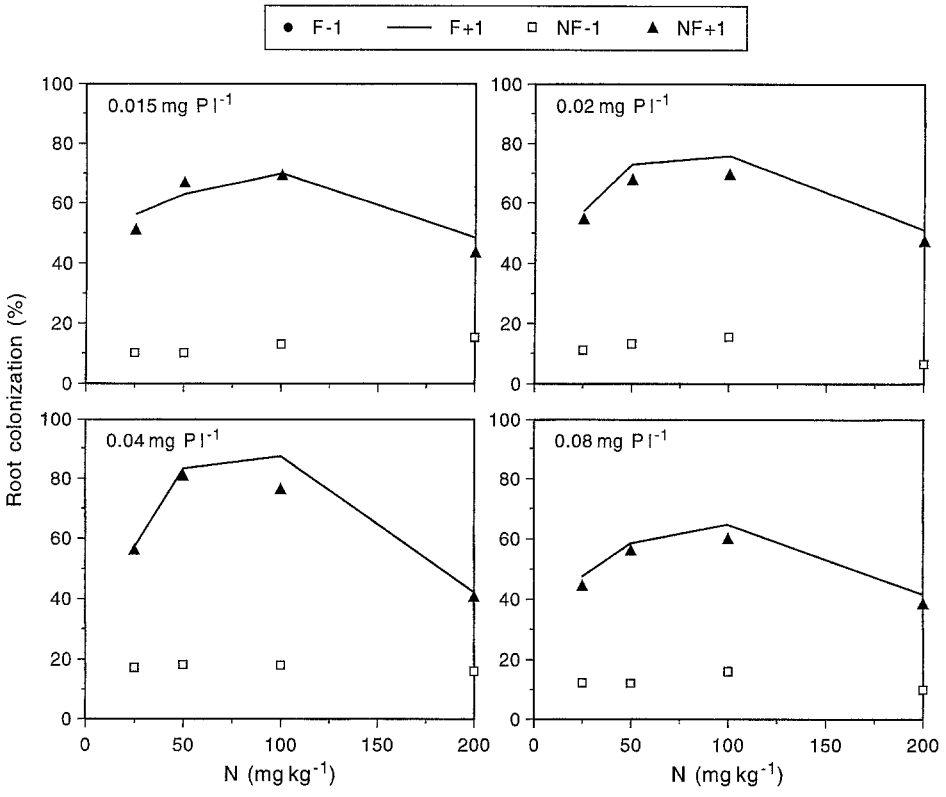

Fig. 1 Effect of pretransplant mycorrhizal inoculation, soil fumigation, added N and soil solution P concentration on VAMF colonization of roots of *Leucaena leucocephala* seedlings after transplanting. $LSD_{.05} = 3.50$. *F* Fumigated, *NF* nonfumigated, *+I* inoculated, *–I* noninoculated

kg^{-1} soil, but colonization levels were lower if the pretransplant growth media were unamended with P (native P content of 0.015 mg l^{-1}) or amended with P to attain 0.08 mg l^{-1} (Fig. 5) than at the other P concentrations. The highest level of VAMF colonization was observed in plants started from seedlings raised in soil amended with 100 mg N kg^{-1} and 0.04 mg P l^{-1}, irrespective of soil fumigation.

Posttransplant VAMF effectiveness

Pretransplant inoculation with *G. aggregatum* significantly increased the posttransplant pinnule P content of *L. leucocephala* at all pretransplant soil P and N levels tested (Figs. 2–5). Pinnule P content increased beginning at 15 DAT and reached maximum levels at 28 DAT. The maximal amount of P observed in pinnules varied with pretransplant N and P amendments and soil fumigation. In the fumigated soil, maximal VAMF inoculation effect was observed if the soil was treated with 100 mg N kg^{-1} and fertilized with P to attain a target P concentration of 0.04 mg l^{-1}. In the nonfumigated soil, maximal inoculation effect was noted if the soil was fertilized with 50 mg N kg^{-1} and amended with P to attain a target concentration of 0.04 mg l^{-1}. Increasing N above 100 mg N kg^{-1} soil and P above 0.04 mg P l^{-1} in the pretransplant soil decreased maximum posttransplant pinnule P content (Fig. 5). However, differences in pinnule P content of inoculated and uninoculated plants started from seedlings raised in the nonsterile soil were generally wider at the latter N and P concentrations than at the initial two increments of N and P. Pinnule P content was generally higher in the fumigated soil than in the nonfumigated soil, but the patterns of response observed in the two soils were similar.

Growth and tissue N and P status

At transplanting, seedlings grown in the uninoculated soils were generally shorter than those raised in the inoculated soils (Table 1). The stature of transplants raised in inoculated soils was similar, irrespective of soil fumigation treatment. Seedlings raised in soil supplied with 0.015 mg P l^{-1} were generally shorter than those grown at other P levels across all N concentrations.

At 20 DAT, transplants raised in the fumigated inoculated soil were generally larger than those raised in the nonfumigated inoculated soil (Table 2). At this stage, transplants raised in the fumigated uninoculated soil became stunted and chlorotic. However, all transplants survived after transplanting, although seedlings precolonized with VAMF appeared to establish relatively rapidly compared with those not colonized by the fungi.

Pretransplant VAMF inoculation significantly increased plant height, shoot dry weight, shoot P and shoot N concentrations, and shoot N:P ratios measured at harvest (Table 3). The extent to which VAMF inoculation increased plant growth and nutrient uptake depended on the levels of N and P fertilizers supplied to the pretransplant growth medium. Average shoot dry

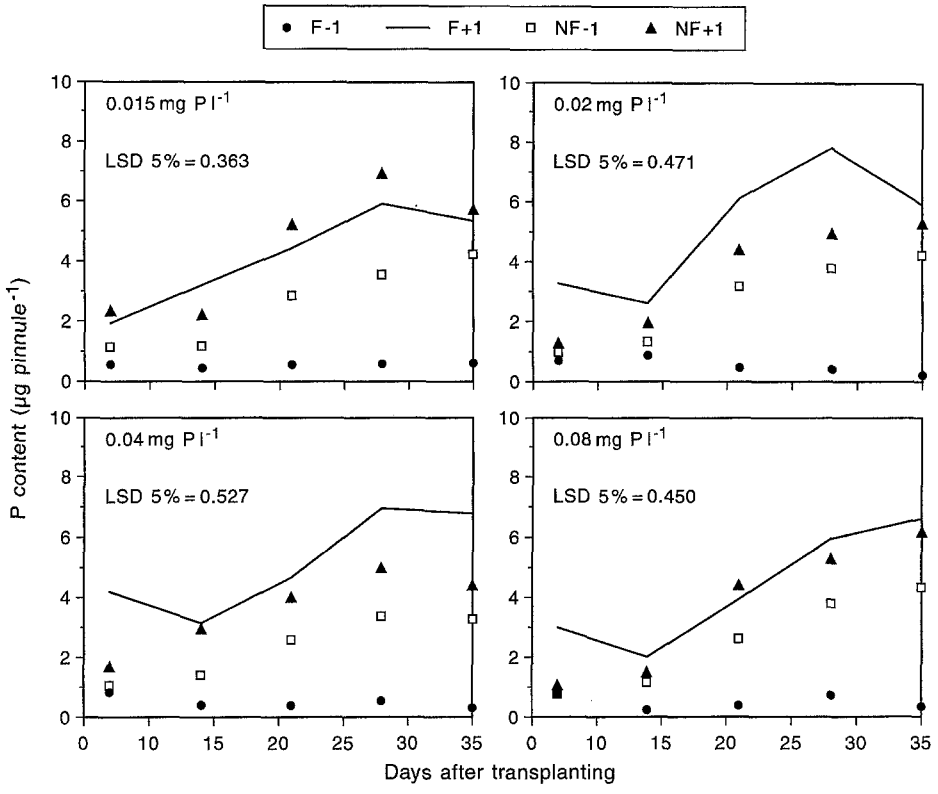

Fig. 2 Effects of pretransplant mycorrhizal inoculation, soil fumigation, and soil solution P concentration on posttransplant VAMF effectiveness at 25 mg N kg^{-1}. Abbreviations as in Fig. 1

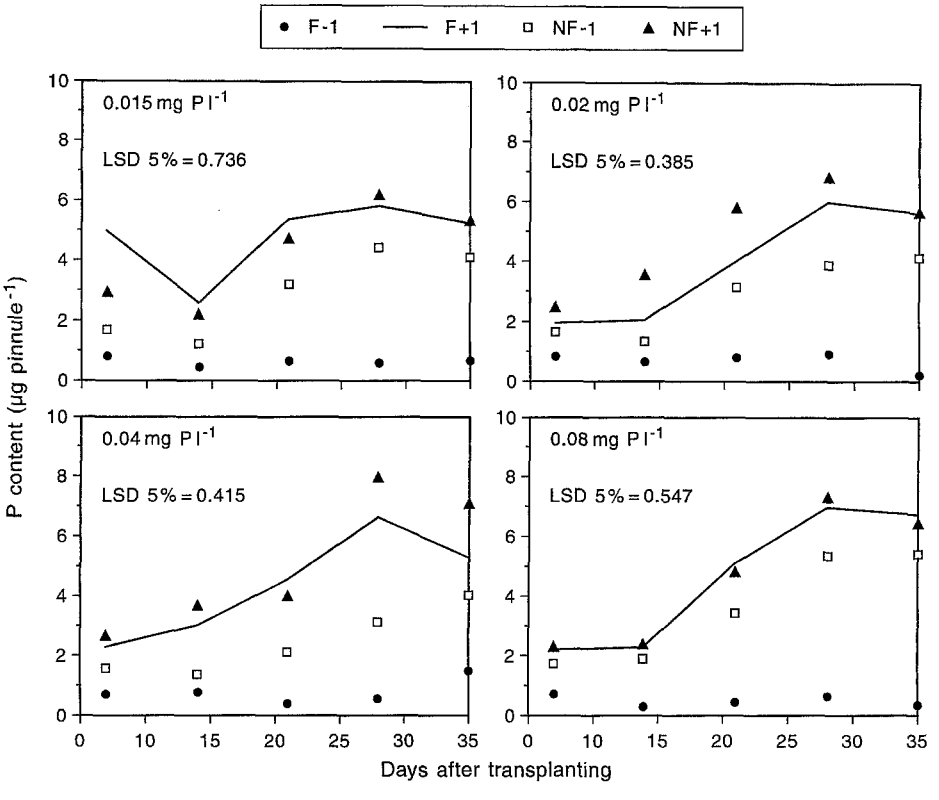

Fig. 3 Effects of pretransplant mycorrhizal inoculation, soil fumigation, and soil solution P concentration on posttransplant VAMF effectiveness at 50 mg N kg^{-1}. Abbreviations as in Fig. 1

weight at 47 DAY was 6.01, 5.35, 2.84, and 0.544 g $plant^{-1}$ for seedlings raised in the fumigated inoculated, nonfumigated inoculated, nonfumigated uninoculated, and fumigated uninoculated soil, respectively. Plant growth variables were significantly higher if the pretransplant growth media were supplied with 0.08 mg P l^{-1} than with 0.015 mg P l^{-1} at all N levels. However, root infection levels (Fig. 1) and growth performance were highest if the non-fumigated inoculated pretransplant medium was amended with 50 mg N kg^{-1} soil and

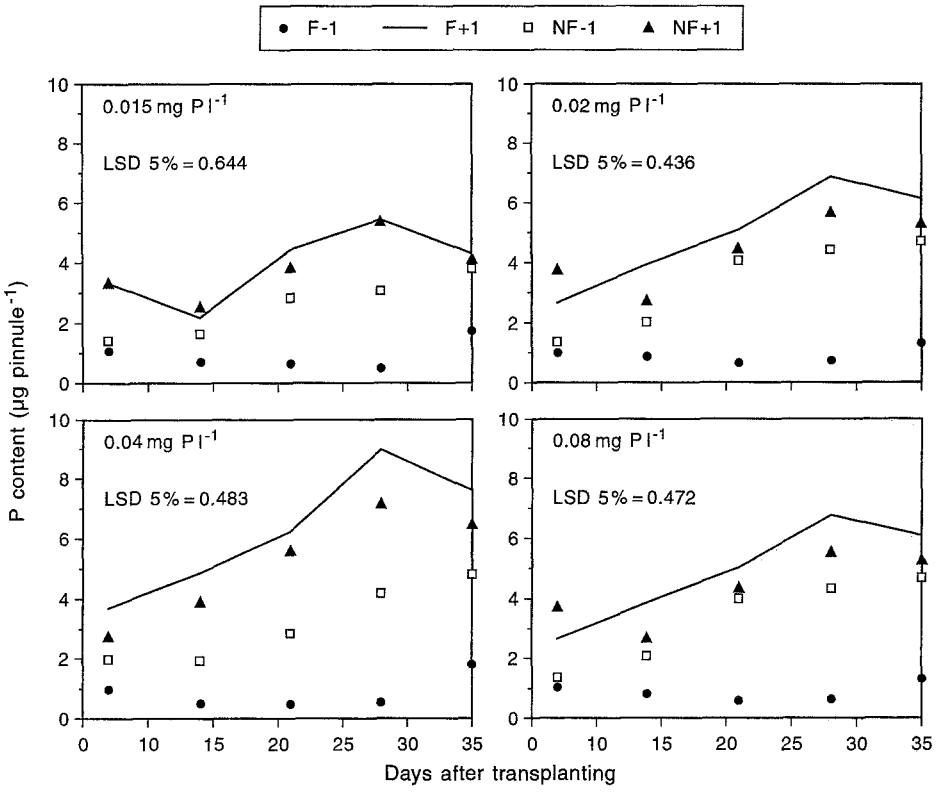

Fig. 4 Effects of pretransplant mycorrhizal inoculation, soil fumigation, and soil solution P concentration on posttransplant VAMF effectiveness at 100 mg N kg^{-1}. Abbreviations as in Fig. 1

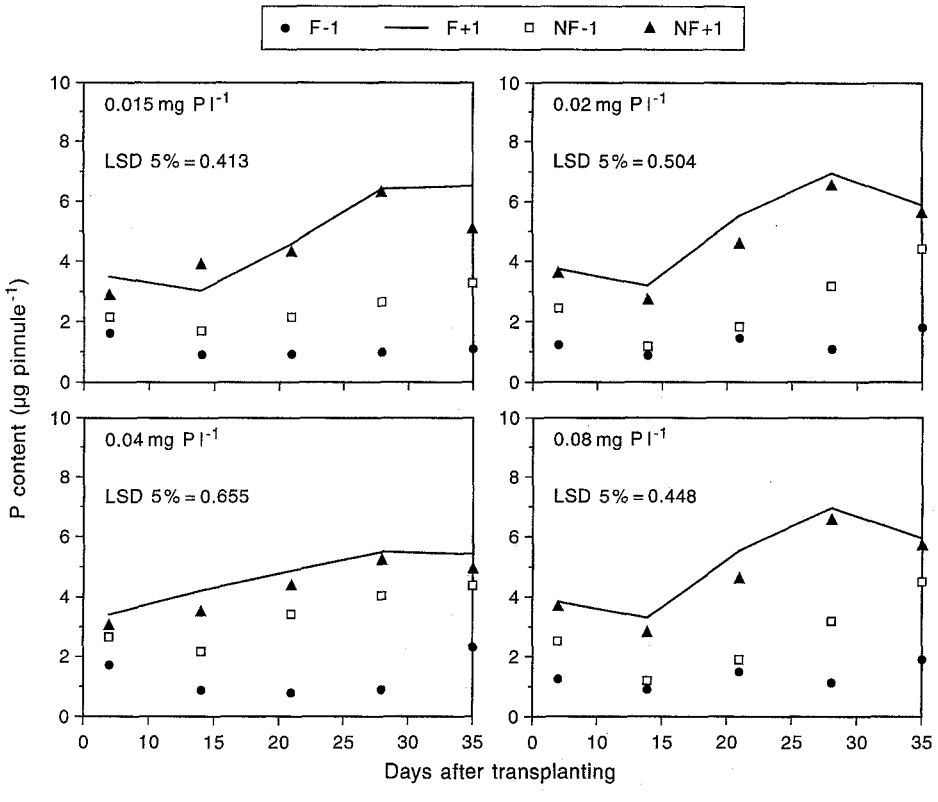

Fig. 5 Effects of pretransplant mycorrhizal inoculation, soil fumigation, and soil solution P concentration on posttransplant VAMF effectiveness at 200 mg N kg^{-1}. Abbreviations as in Fig. 1

0.04 mg P l^{-1} or if the medium was fumigated and supplied with 100 mg N kg^{-1} and 0.04 mg P l^{-1}. The highest growth performance of seedlings raised in the uninoculated nonfumigated soil was observed when the soil was amended with 100 mg N kg^{-1} soil and 0.08 mg P l^{-1} and when the fumigated medium was amended with 200 mg N kg^{-1} soil and 0.08 mg P l^{-1} (Figs. 6–8, Table 4).

Maximal tissue N and P accumulation was observed in the fumigated inoculated soil when it was supplied with 100 mg N kg^{-1} and 0.04 mg P l^{-1}. The next highest

Table 1 Heights (cm) of *Leucaena leucocephala* seedlings at transplanting as influenced by pretransplant mycorrhizal inoculation, soil fumigation, and N and P fertilization (average of triplicates) (*NF−I* non-fumigated uninoculated, *NF+I* non-fumigated inoculated; *F−I* fumigated uninoculated, *F+I* fumigated inoculated). $LSD_{0.05}=0.974$

P ($mg\ l^{-1}$)	N ($mg\ kg^{-1}$)	NF−I	NF+I	F−I	F+I	Average
0.015	25	6.6	7.6	5.0	9.6	7.2
	50	7.8	8.0	6.0	10.8	8.1
	100	7.8	8.9	5.0	11.7	8.3
	200	6.2	7.6	5.9	10.3	7.5
0.02	25	9.3	13.9	7.0	14.0	11.0
	50	10.4	15.5	6.0	15.0	11.7
	100	11.7	17.7	8.6	16.0	13.5
	200	11.7	14.9	7.1	14.1	11.9
0.04	25	11.5	15.1	8.0	13.9	12.1
	50	12.6	15.9	6.0	15.3	12.4
	100	12.6	16.7	7.7	16.0	13.2
	200	10.2	15.8	8.0	14.1	12.0
0.08	25	10.9	13.9	8.0	11.7	11.1
	50	10.9	15.8	8.6	14.2	12.4
	100	11.8	15.1	9.3	15.9	13.0
	200	10.1	14.4	9.7	13.1	11.8

Table 2 Plant heights (cm) 21 days after transplanting as influenced by pretransplant mycorrhizal inoculation, N and P levels, and soil fumigation (average of triplicates). Abbreviations as in Table 1. $LSD_{0.05}=1.71$

P ($mg\ l^{-1}$)	N ($mg\ kg^{-1}$)	NF−I	NF+I	F−I	F+I	Average
0.015	25	8.4	9.5	6.9	11.8	9.1
	50	8.9	9.7	7.2	12.8	9.6
	100	8.9	10.8	7.2	13.0	10.0
	200	8.3	8.8	8.1	12.0	9.3
0.02	25	11.2	16.5	7.2	17.4	13.1
	50	12.1	17.1	8.2	19.0	14.1
	100	13.1	17.4	8.7	18.9	14.5
	200	13.0	16.1	9.1	18.7	14.2
0.04	25	12.0	16.5	7.6	17.5	13.4
	50	13.1	18.7	8.5	19.1	14.8
	100	14.8	17.4	9.7	19.3	15.3
	200	13.3	17.2	10.0	17.4	14.5
0.08	25	12.6	15.0	9.3	15.9	12.8
	50	13.4	17.0	10.6	17.8	14.7
	100	15.7	17.4	10.7	18.4	15.5
	200	12.6	17.1	11.7	17.2	14.6

Table 3 *F*-test significance for plant heights, shoot dry weights, shoot phosphorus and nitrogen concentrations, shoot N:P ratios, and percent root infection of *L. leucocephala* seedlings 47 days after transplanting as influenced by pretransplant mycorrhizal inoculation, N and P fertilization and soil fumigation (*PRI* percent root infection, *SDW* shoot dry weight, * significant at $P<0.05$, *NS* not significant at $P<0.05$)

Sources of variation	Plant height (cm)	SDW ($g\ plant^{-1}$)	Shoot P (%)	Shoot N (%)	Shoot N:P (%)	PRI (%)
Nitrogen (N)	*	NS	*	*	*	NS
Phosphorus (P)	*	*	*	*	*	*
Inoculation (I)	*	*	*	*	*	*
N×P	NS	*	*	*	NS	NS
N×P×I	*	*	*	*	*	*

Table 4 Plant heights (cm) observed 47 DAT as affected by pretransplant VAMF inoculation, N and P levels, and soil fumigation (average of triplicates). Abbreviations as in Table 1. $LSD_{0.05}=3.47$

P ($mg\ l^{-1}$)	N ($mg\ kg^{-1}$)	NF−I	NF+I	F−I	F+I	Average
0.015	25	24.9	34.8	7.8	38.6	26.5
	50	25.9	39.5	8.1	44.3	29.4
	100	26.3	47.3	8.9	47.7	32.5
	200	29.8	42.1	9.6	42.1	31.9
0.02	25	26.8	44.0	9.3	47.2	31.8
	50	25.9	47.6	9.9	57.2	35.1
	100	26.8	57.2	9.9	58.1	38.0
	200	28.8	49.2	10.5	55.8	36.1
0.04	25	25.4	46.6	11.1	48.6	32.9
	50	30.7	65.5	12.5	66.4	43.8
	100	31.6	60.7	14.9	70.1	44.3
	200	34.2	59.2	15.8	60.5	42.4
0.08	25	26.8	46.1	15.2	56.3	35.8
	50	36.6	51.5	16.0	63.2	41.8
	100	39.5	50.2	17.1	57.0	40.9
	200	31.1	49.4	18.5	55.6	38.6

value was observed in seedlings raised in the nonfumigated inoculated soil amended with 50 mg N kg^{-1} and 0.04 mg P l^{-1}. The extent of tissue N and P accumulation observed can be represented in the following series: Fumigated inoculated > nonfumigated inoculated > nonfumigated uninoculated > fumigated uninoculated.

Shoot N:P ratio after transplanting

The largest shoot N:P ratio was observed in plants which were started from seedlings raised in the fumigated uninoculated soil while the lowest shoot N:P ratio was observed in plants started from seedlings in the fumigated inoculated soil (Fig. 9). When plants were raised in the nonfumigated soil, shoot N:P ratios ranged from 14 to 34.

No relationship was found between shoot N:P ratio and the N:P ratio in the nutrients added to the pretransplanting growth medium (Fig. 9). There was also

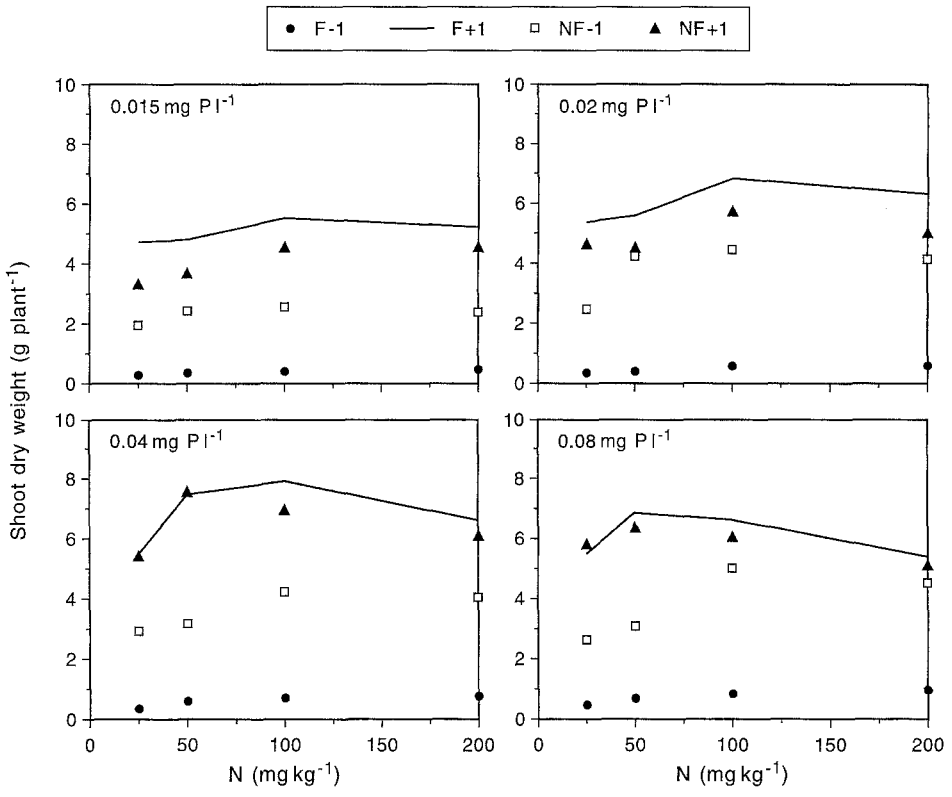

Fig. 6 Effects of pretransplant mycorrhizal inoculation, soil fumigation, added N and soil solution P concentration on posttransplant shoot dry weight of *L. leucocephala*. $LSD_{.05} = 0.586$. Abbreviations as in Fig. 1

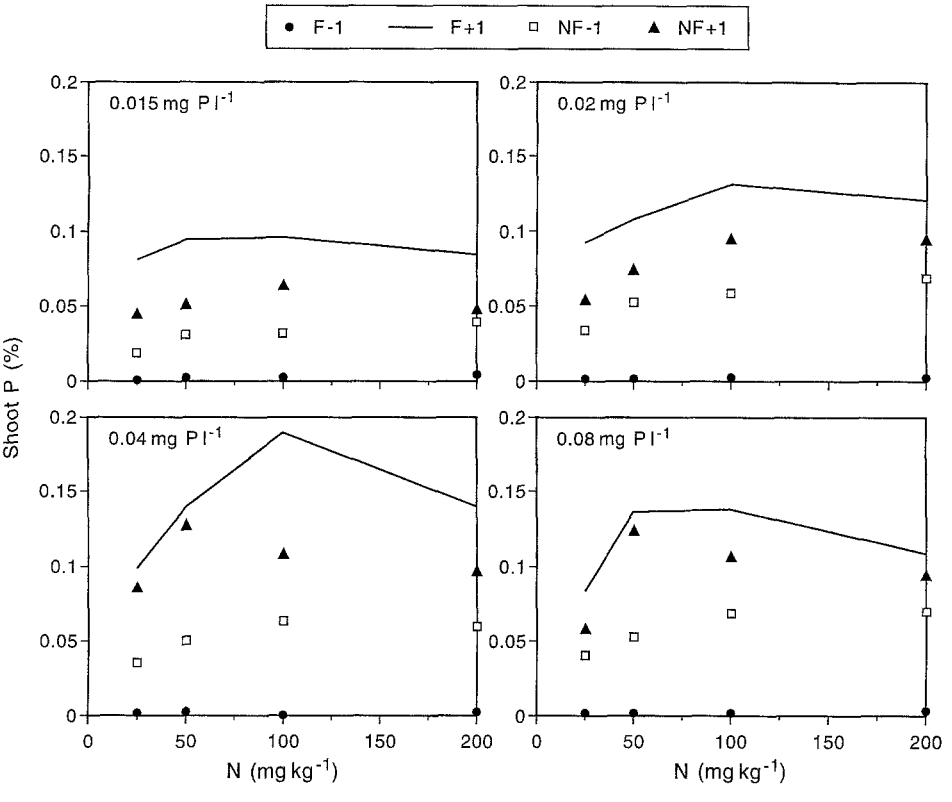

Fig. 7 Effects of pretransplant mycorrhizal inoculation, soil fumigation, added N and soil solution P concentration on posttransplant shoot P concentration of *L. leucocephala*. $LSD_{.05} = 0.0057$. Abbreviations as in Fig. 1

no relationship between root colonization and shoot N:P ratio of plants whose seedlings were raised in the inoculated soil. However, their shoot dry weights were negatively correlated with shoot N:P ratios (Fig. 10). Shoot dry weights of plants raised in the fumigated uninoculated pretransplant soil were positively correlated with shoot N:P ratios ($r = 0.684$, $P < 0.05$), but there was no relationship between shoot dry weight and shoot N:P ratios ($r = -0.373$, NS) if seedlings were raised in the nonfumigated uninoculated pretransplant soil.

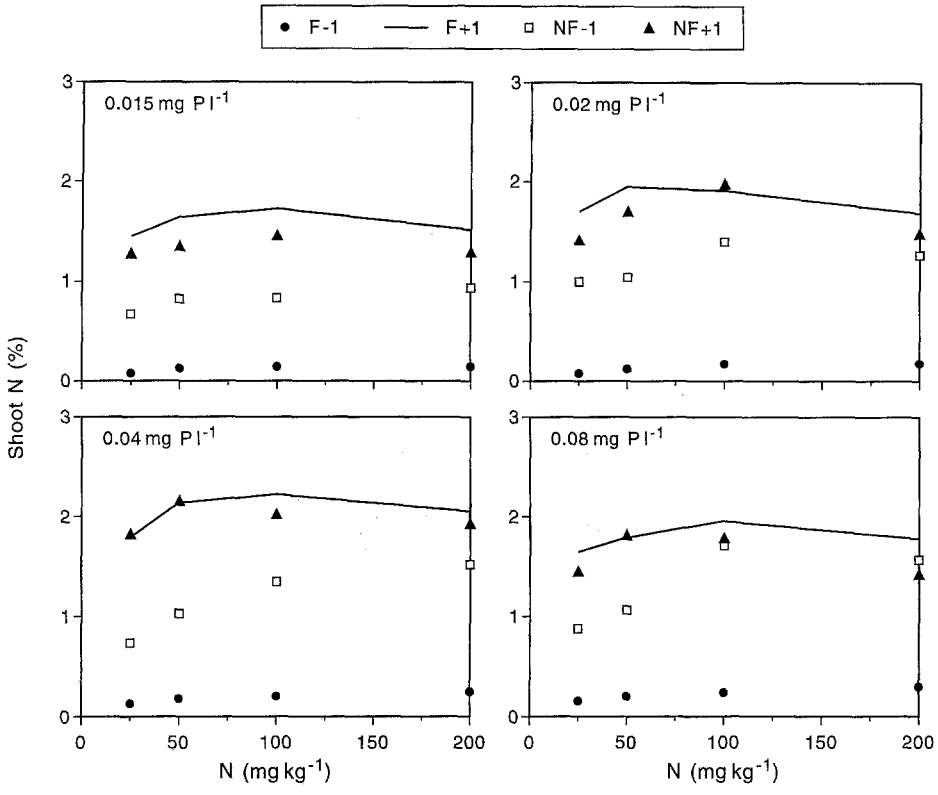

Fig. 8 Effects of pretransplant mycorrhizal inoculation, soil fumigation, added N and soil solution P concentration on posttransplant shoot nitrogen concentration of *L. leucocephala*. $LSD_{.05} = 0.113$. Abbreviations as in Fig. 1

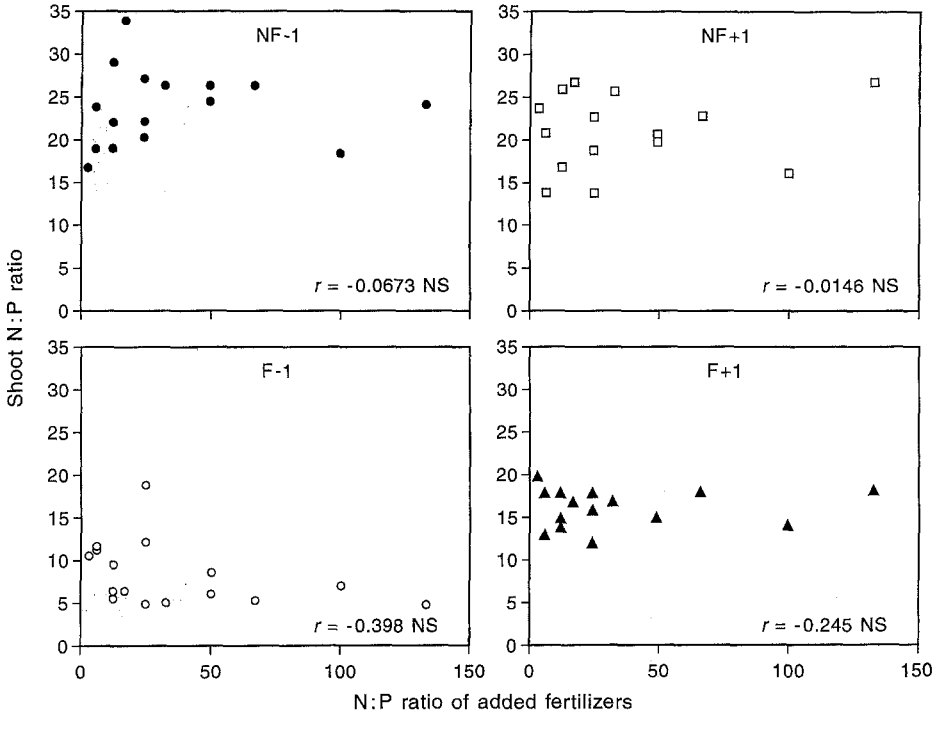

Fig. 9 Relationship between posttransplant shoot N:P ratios and N:P ratios of nutrients added to the pretransplant medium. The values shown are 1% of the actual values. Abbreviations as in Fig. 1. *NS* Not significant at $P < 0.05$

Discussion

VAMF colonization of roots after transplanting

The posttransplanting VAMF colonization level of seedlings raised in the non-fumigated uninoculated pretransplanting soil was low (Fig. 1), probably because of the reduction in indigenous VAMF propagules caused by the removal of the surface soil (Habte 1989). The similarity in posttransplant root colonization levels of seedlings raised in sterile and nonsterile soils suggests that the introduced endophyte was able to colonize roots in the nonfumigated soil as readily as in the fumigated soil.

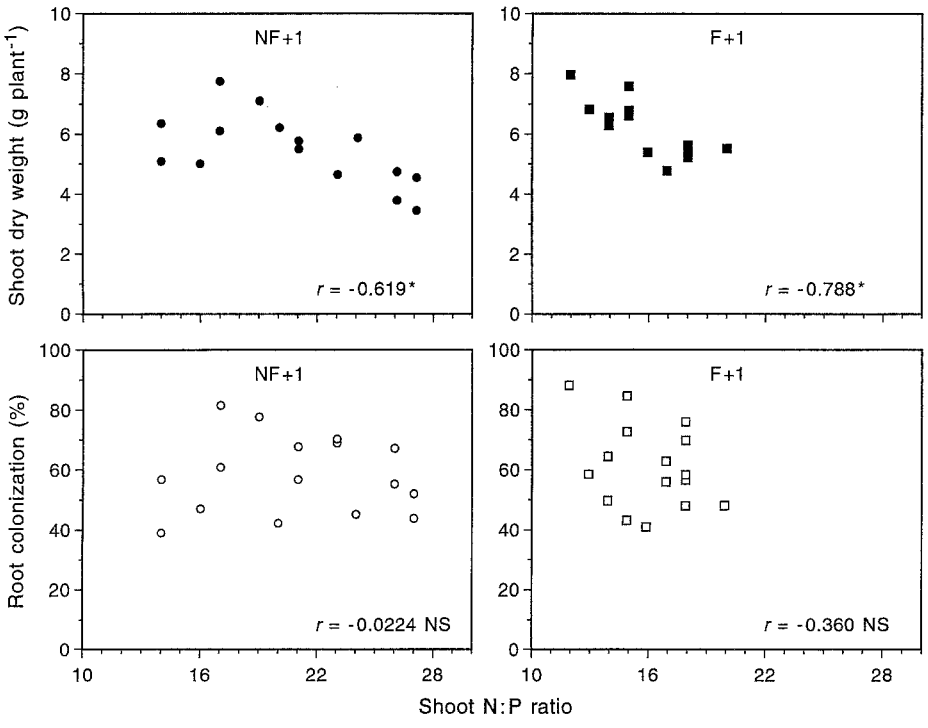

Fig. 10 Relationships between shoot N:P ratios and shoot dry weight after transplanting, and shoot N:P ratios and VAMF colonization of roots of *Leucaena* after transplanting. Data are derived from mycorrhizal plants raised in the fumigated inoculated pretransplant medium ($F+I$) and the non- fumigated inoculated medium ($NF+I$). * Significant at $P<0.05$, *NS* not signifikant

N and P required for raising VAMF-infected seedlings

Seedlings need adequate amounts of nutrients for rapid and vigorous early growth. However, the production of VAMF-colonized seedlings requires a compromise between supplying adequate nutrients for vigorous growth of seedlings and maintaining nutrient levels low enough to promote maximal colonization of roots by VAMF. In our study, plant growth parameters measured at the end of the experiment were lower if the seedlings were raised in the pretransplant soils amended with 0.015 mg P l^{-1} than with 0.08 mg P l^{-1}, irrespective of the levels of pretransplant N applied (Table 4, Figs. 6–8). On the other hand, the levels of VAMF colonization observed when the pretransplant soil was supplied with 0.015 mg P l^{-1} were significantly higher than those observed when soil P was adjusted to 0.08 mg l^{-1} (Fig. 1). However, the former colonization levels were significantly lower than those observed at a target soil solution P concentration of 0.04 mg l^{-1} at all N levels (Fig. 5), suggesting an optimal P concentration of 0.04 mg l^{-1} for pretransplant mycorrhization of seedlings. Indeed the best mycorrhizal growth responses and the highest root colonization levels were observed at 50 mg N kg^{-1} soil and 0.04 mg P l^{-1} if the inoculated pretransplant soil was not fumigated or 100 mg N kg^{-1} soil and 0.04 mg P l^{-1} if the inoculated pretransplant soil was fumigated (Figs. 1, 5, 6). This was true also of tissue N and P concentrations (Figs. 7, 8). The shoot dry weight of plants increased by 46% and 91% as a result of precolonization of seedling by *G. aggregatum* in non-fumigated and fumigated soils, respectively. The pretransplant fertilizer amendments required to achieve maximal posttransplant shoot dry weight were half as much if seedlings were precolonized by VAMF as if they were not.

The lower N requirement of mycorrhizal seedlings raised in the nonfumigated soil compared with those raised in the fumigated one is perhaps due to the contribution of biological nitrogen fixation to N nutrition in the former case. Seedlings raised in the former medium were nodulated, while those raised in the fumigated soil were not.

Hepper (1983) suggested that the N:P ratio in nutrient solutions may be the most important factor governing mycorrhizal colonization. By adding nutrient solutions twice weekly in a sand:grit mixture, she observed that colonization of lettuce (*Lactuca sativa*) roots by *Glomus mosseae* decreased when the N:P ratio of the nutrient solution exceeded 15.

Tissue N:P ratios may indicate the adequacy or inadequacy of nutrients for normal functioning of an organism in a given environment (Reeves 1987). In plants growing under "sufficient" nutrient conditions, the N content of green plant material is about 10 times higher than that of P (Mengel and Kirby 1982). Thus tissue N:P ratios greater than 10 may indicate P inadequacy, while N:P ratios less than 10 may imply N adequacy. However, in light of the fact that shoot N:P ratios of plants started from mycorrhizal seedlings were negatively correlated with shoot dry weight (Fig. 9), the value of N:P ratios as indicators of nutrient sufficiency or inadequacy is not clear. We also found no relationship between root colonization and shoot N:P ratio (Fig. 10). Our findings concur with those of Sylvia and Neal (1990), who observed no relationship between tis-

sue N:P ratio of *Allium cepa* and root colonization by two isolates of *Glomus etunicatum* in a fine sandy loam (grossarenic paleudult).

Our results suggest that the posttransplant performance of seedlings colonized by VAMF could be enhanced significantly through proper management of N and P content of the pretransplant soil.

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References

Azcon R, Gomez-Ortega M, Barea JM (1982) Comparative effects of foliar- or soil-applied nitrate on vesicular-arbuscular mycorrhizal infection in maize. New Phytol 92:553–559

Aziz T, Habte M (1990) Enhancement of endomycorrhizal activity through nitrogen fertilization in cowpea grown in an oxisol subjected to simulated erosion. Arid Soil Res Rehab 4:131–139

Bolan NS, Robson AD, Barrow NJ (1984) Increasing phosphorus supply can increase the colonization of plants by vesicular-arbuscular mycorrhizal fungi. Soil Biol Biochem 16:419–489

Chapman HD, Pratt PF (1961) Methods of analysis for soils, plants, and waters. (Publication no 4034) Division of Agricultural Sciences, University of California, Berkeley, Calif

Cornet F, Diem HG, Dommergues YR (1982) Effect de l'inoculation avec *Glomus mosseae* sur la croissance d'*Acacia holoserica* en pepiniere et apres transplantation sur le terrain. In: Gianinazzi S, Gianinazzi-Pearson V, Trouvelot A (eds) Mycorrhizas, an integral part of plants' biology: prospects for their use. INRA, Paris, pp 287–293

Fox RL, Kamprath EJ (1970) Phosphate sorption isotherms for evaluating P requirements of soils. Soil Sci Soc Am J 34:902–907

Giovanetti JW, Mosse B (1980) An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Habte M (1992) The usefulness of the pinnule technique in mycorrhizal research. Methods Microbiol 24:323–338

Habte M, Manjunath A (1987) Soil solution P status and mycorrhizal dependency in *Leucaena leucocephala*. Appl Environ Microbiol 53:797–801

Hall IR, Johnstone PD, Dolby R (1984) Interactions between endomycorrhas and soil N and P on the growth of ryegrass. New Phytol 97:447–453

Hayman DS (1982) Influence of soils and fertility on activity and survival of vesicular-arbuscular mycorrhizal fungi. Am Phytopathol Soc 72:1119–1125

Hepper CM (1983) The effect of nitrate and phosphate on the vesicular-arbuscular mycorrhizal infection of lettuce. New Phytol 93:389–399

Kormanik PP, Bryan WC, Schultz RC (1980) Procedure and equipment for staining large numbers of plant root samples for endomycorrhizal assay. Can J Microbiol 26:536–538

Kruckelman HW (1975) Effects of fertilizers, soils, soil tillage and plant species on the frequency of *Endogone* chlamydospores and mycorrhizal infection in arable soils. In: Sanders FE, Mosse B, Tinker PB (eds) Endomycorrhizas. Academic Press, New York, pp 511–525

Menge JA, Sterile D, Bagyaraj DJ, Johnson ELV, Leonard RT (1978b) Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. New Phytol 80:575–578

Mengel K, Kirby EA (1982) Principles of plant nutrition, 3rd edn. Bund AG, Bern

Miranda JCC, Harris PJ, Wild A (1989) Effects of soil and plant phosphorus concentrations on vesicular-arbuscular mycorrhiza in sorghum plants. New Phytol 112:405–410

Mosse B (1973) Plant growth responses to vesicular-arbuscular mycorrhiza in soil given additional phosphate. New Phytol 72:127–136

Murphy J, Riley JP (1962) A modified single method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–35

Plenchette C, Fortin JA, Furlan V (1983) Growth stimulation of apple trees in unsterilized soil under field conditions with VAM mycorrhiza inoculation. Can J Bot 59:2003–2008

Reeves BF (1987) Mycorrhizal responsiveness and ecology of VAM in arid and semiarid ecosystems. In: Sylvia DM, Hung LL, Graham J (eds) Mycorrhizas in the next decade. Proceedings of the 7th North American Conference on Mycorrhizas, University of Florida, Gainesville, Fla, p 136

SAS Institute (1985) SAS user's guide: statistics. SAS Institute Inc, Cary, NC

Sasa M, Zahka G, Jakobsen I (1987) The effect of pretransplant inoculation with VA mycorrhizal fungi on the subsequent growth of leeks in the field. Plant Soil 97:279–283

Sreeramulu KR, Bagyaraj DJ (1986) Field response of chilli to VA mycorrhiza on black clayey soil. Plant Soil 93:299–3021

Sylvia DM, Neal LH (1990) Nitrogen affects the phosphorus response of VA mycorrhiza. New Phytol 115:303–310

Vinayak K, Bagyaraj DJ (1990) Selection of efficient VA mycorrhizal fungi for trifoliate orange. Biol Agric Hortic 6:305–311