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Categories of vesicular-arbuscular mycorrhizal dependency of host species*

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Summary. *Brassica nigra* and selected species of *Leucaena* and *Sesbania* were used as indicator hosts in a greenhouse experiment designed to establish distinct categories of mycorrhizal dependence. The plants were grown in an oxisol with different concentrations of established soil solution P in the presence or absence of the vesicular-arbuscular mycorrhizal (VAM) fungus *Glomus aggregatum*. The extent to which the plant species depended on the fungus for dry matter production diminished with increased concentrations of soil solution P, but the magnitude of this decrease varied from species to species. Five distinct mycorrhizal categories are proposed based on the differences observed, ranging from non-dependent to very highly dependent. The critical soil solution P concentrations that were useful for separating host species into distinct VAM-dependency groups were 0.02 and 0.2 mg/l. Species differing in their mycorrhizal dependency differed with respect to the soil solution P concentration required for the expression of maximum VAM effectiveness, the degree to which increasing concentrations of P depressed VAM infection and the pattern of immobile nutrient accumulation.

Key words: *Brassica nigra* – Established P – *Glomus aggregatum* – *Leucaena* species – *Sesbania* species

Introduction

More than a decade ago, the term "mycorrhizal dependency" (MD) was defined as the degree to which a plant species is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility (Gerdemann 1975). Since then, numerous attempts have been made to determine the MD of several plant species (Azcon and Ocampo 1981; Hetrick et al. 1988; Nemec 1971; Plenchette et al. 1983; Pope et al. 1983; Saif 1987). The above investigations were conducted either at native soil solution P concentrations or in soils to which nutrient solution at one concentration of P or rock phosphate was added. It may not be possible to differentiate host species into distinct vesicular-arbuscular mycorrhizal (VAM) dependency categories if the effect of VAM infection is evaluated using a soil to which a single P concentration is added. A plant species characterized as having a particular degree of VAM dependency in one soil is bound to have an entirely different degree of VAM dependency in another soil depending on the P adsorbing capacity of the soils, and hence on the concentration of P attained in the soil solution (Aziz and Habte 1987; Habte and Manjunath 1987). Therefore, a clear evaluation of the VAM dependency of plant species is best accomplished when VAM fungi and host species are allowed to interact across a gradient of established soil solution P concentrations.

Knowledge of the VAM dependency of host species is important because the extent to which a host plant will respond to VAM infection is likely to be a function of its VAM dependency and this is necessary for predicting host response to VAM inoculation.

The objective of the current investigation was to establish distinct VAM-dependency categories of host species using an oxisol with established soil solution P concentrations, selected species of *Leucaena* and *Sesbania* as indicator hosts and *Brassica nigra* as a nonmycorrhizal reference species.

Materials and methods

Soil preparation and treatment

The soil used in this investigation was a subsurface sample (15–30 cm) of an oxisol (Tropeptic Eutrustox, Wahiawa series) with a pH of 5.4 (1:2 soil-water suspension). A subsoil was selected because of the low soil solution P concentration (0.002 mg/l), and to minimize the influence of organic P on P availability. The soil was crushed to pass through a 4-mm sieve. The pH of the soil was

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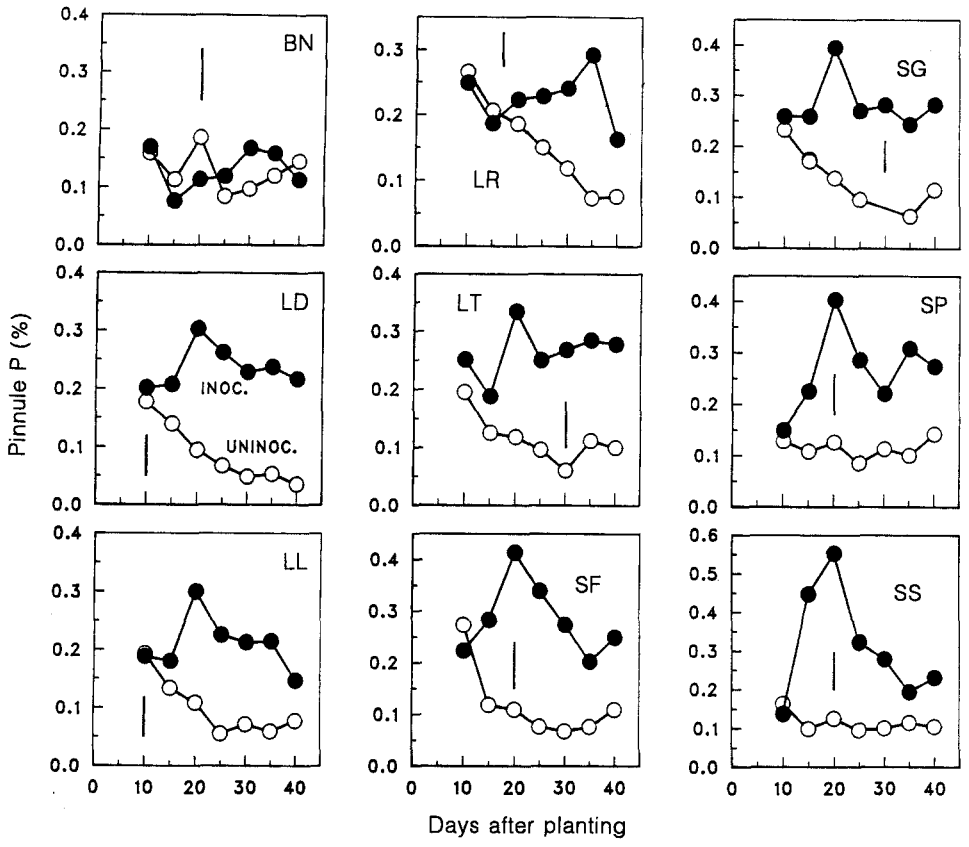

Fig. 1. P concentration in leaf disks of *Brassica nigra* and in pinnules of *Leucaena* and *Sesbania* species as influenced by inoculation with *Glomus aggregatum* at soil solution P concentration of 0.002 mg/l. *Vertical bars* represent least significant difference (LSD) values at the 5% level. *INOC.*, Inoculated with *G. aggregatum* (●); *UNINOC.*, uninoculated (○); *BN*, *B. nigra*; *LD*, *Leucaena diversifolia*; *LL*, *L. leucocephala*; *LR*, *L. retusa*; *LT*, *L. trichodes*; *SF*, *Sesbania formosa*; *SG*, *S. grandiflora*; *SP*, *S. pachycarpa*; *SS*, *S. sesban*

adjusted to 6.0 using $Ca(OH)_2$ (1.14 g/kg soil) to eliminate the potential adverse effect of Mn. Portions (2.5 kg; dry weight basis) of the soil were then transferred into plastic pots (15 × 18 cm).

In addition to the initial P concentration of the soil (0.002 mg/l), two concentrations of soil solution, namely 0.02 and 0.2 mg/l, were established as described by Manjunath and Habte (1990). The middle level has been demonstrated to be optimal for VAM activity, at least in *Leucaena leucocephala* and *Vigna unguiculata* (Aziz and Habte 1987; Habte and Manjunath 1987), and 0.2 mg P/l is a level which produces 95% of maximum yield in many crop species (Fox 1981). Nitrogen was added as $Mg(NO_3)_2 \cdot 6H_2O$ at the rate of 34.6 mg N/kg soil. Other essential nutrients were added as described previously (Habte and Manjunath 1987). The pots containing soil were fumigated with 48 g methyl bromide and 1 g chloropicrin in a gas-tight chamber for 4 days on two separate occasions (Manjunath and Habte 1980). The pots were removed from the chamber, placed on greenhouse benches and left to stand for 15 days for the fumigants to dissipate.

Mycorrhizal inoculation

Mycorrhizal inoculation of soils in some of the pots was carried out by mixing the contents of each pot with 50 g of a 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 50 g sterilized sand and washings of the crude inoculum after removal of *G. aggregatum* structures by filtration (Whatman no. 1 filter paper). The crude inoculum of *G. aggregatum* was prepared as described by Habte and Manjunath (1987).

Seed treatment and planting

Seeds of *Leucaena diversifolia* (Schlecht.) Benth., *L. leucocephala* (Lam.) de Wit, *L. retusa* Benth., *L. trichodes* (Jacq.) Benth., *Sesbania formosa* (Muell.) Burbi., *S. grandiflora* (L.) Poir., *S. pachycarpa* DC., Prodr. and *S. sesban* (L.) Merr. were pregerminated on water agar (0.9%) after scarification with concentrated H_2SO_4. The scarification time for *L. diversifolia*, *L. leucocephala*, *L. retusa*, *L. trichodes* and *S. grandiflora* was 20 min. Seeds of *S. formosa*, *S. pachycarpa* and *S. sesban* were scarified for 40 min. *Brassica nigra* (L.) Koch. served as a nonmycorrhizal control. Four pregerminated seeds were planted per pot. *B. nigra* seeds were directly sown after surface sterilization. Surface sterilization was achieved by treating seeds with 80% alcohol for 1 min followed by sodium hypochlorite (1.6% available chlorine) for 10 min and washing 6 times with sterile deionized water. After emergence, seedlings were thinned to two plants per pot. There were 54 treatments resulting from factorial combinations of two levels of inoculation, nine plant species and three soil solution P concentrations. Pots were arranged on greenhouse benches in a randomized complete block design with three replicates per treatment. Plants were grown under natural light (21° 19′N and 157° 58′W). Water was added as needed to maintain the growth medium at approximately 60% of maximum water holding capacity.

Measurements taken

The development of VAM effectiveness was monitored by measuring the P concentration of pinnules of *Leucaena* and *Sesbania* species and leaf disk of the youngest fully expanded leaf of *B. nigra* at 5-day intervals, starting on the 10th day after planting (Aziz and Habte 1981; Habte et al. 1987). Plants were harvested after 45 days of growth. Dry weight was determined after drying roots and shoots at 70° C for 48 h. The proportion of roots colonized by VAM fungus was determined by the grid-line intersection method (Giovannetti and Mosse 1980) after clearing roots with 10% KOH and staining with acid fuchsin (0.15% in a lactic acid-glycerol solution) (Kormanik et al. 1980). P contents in plant samples were determined by the molybdate blue method (Murphy and Riley 1962) after dry-ashing. Cu and Zn contents of shoots were determined by atomic absorption spectroscopy and K con-

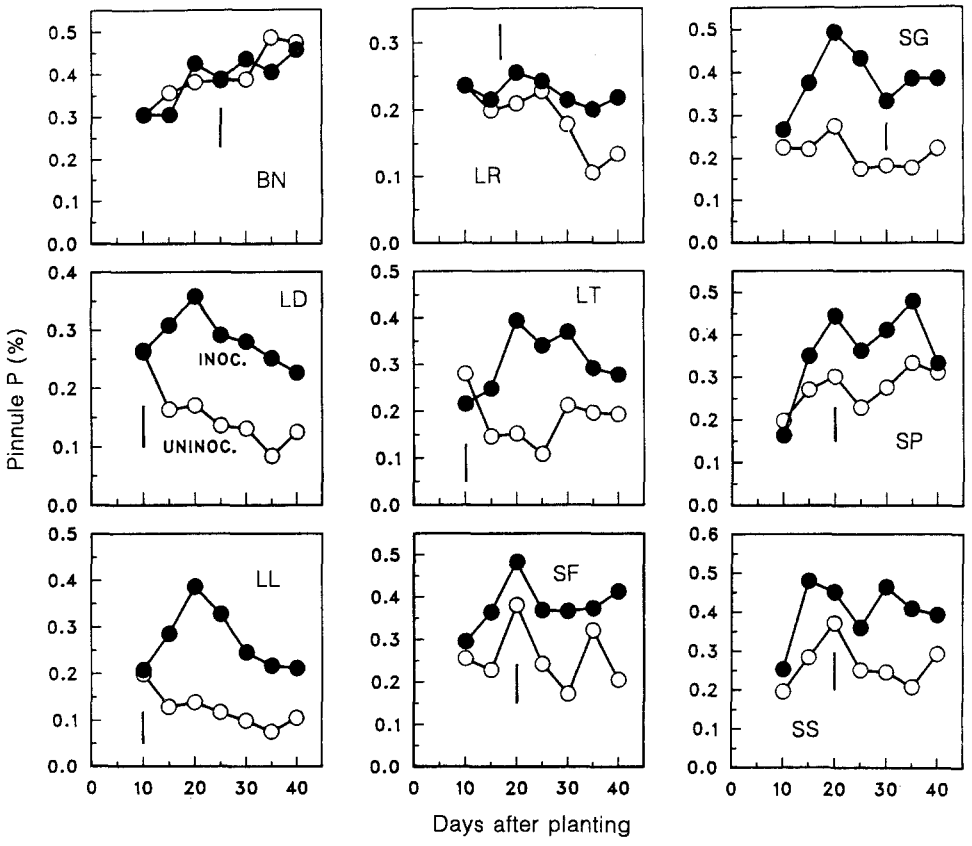

Fig. 2. P concentration in leaf disks of *B. nigra* and in pinnules of *Leucaena* and *Sesbania* species as influenced by inoculation with *G. aggregatum* at a soil solution P concentration of 0.02 mg/l. *Vertical bars* represent LSD values at the 5% level. *INOC.*, Inoculated with *G. aggregatum* (●); *UNINOC.*, uninoculated (○). Species abbreviations are the same as in Fig. 1

tents in shoots were determined by atomic emission spectroscopy (Hue and Evans 1985). MD is expressed as the difference between total dry matter yields of inoculated and uninoculated plants as a percentage of total dry matter yield of inoculated plants (Plenchette et al. 1983). Analysis of data was carried out using the SAS (Statistical Analysis System) procedure (SAS Institute 1982).

Results

Development of VAM effectiveness

Inoculation of soil with *G. aggregatum* did not influence the P concentration of leaf disks of *B. nigra* at any concentration of soil solution P tested. Mycorrhizal inoculation significantly increased the P concentration of pinnules of *Leucaena* and *Sesbania* species (Fig. 1). Significant VAM activity was noticed in all *Sesbania* species and in *L. diversifolia* as early as 15 days after planting (DAP), whereas in *L. leucocephala and L. trichodes* it was observed 20 DAP and in *L. retusa* 25 DAP. The P concentration in pinnules of nonmycorrhizal plants decreased with time except in *S. pachycarpa* and *S. sesban*, which maintained a pinnule P concentration of approximately 0.1% throughout the study period.

Significant VAM activity was also observed in *Leucaena* and *Sesbania* species at a soil solution P concentration of 0.02 mg/l (Fig. 2). Differences in pinnule P concentration between mycorrhizal and nonmycorrhizal *L. diversifolia*, *L. leucocephala*, *L. trichodes* and *S. grandiflora* were significant beginning 15 DAP. The response observed in *S. formosa*, *S. pachycarpa* and *S. sesban* were similar but less uniform. Mycorrhizal and nonmycorrhizal *L. retusa* did not differ in pinnule P content until 35 DAP. At the peak period of VAM activity, the P concentrations of pinnules of *Sesbania* species were higher than those of *Leucaena* species. Increase in soil solution P concentration from 0.002 to 0.02 mg/l resulted in a significant increase in the pinnule P concentration of nonmycorrhizal *Sesbania*, but not of *Leucaena* species. At a soil solution P concentration of 0.2 mg/l, VAM inoculation had a significant effect only on the pinnule P concentration of *L. leucocephala and L. trichodes* (Fig. 3). In general, P concentrations of pinnules of *Sesbania* species were higher than those of *Leucaena* species, regardless of soil solution P status and VAM inoculation.

VAM colonization

There was no evidence of VAM infection on roots of *B. nigra*. Among *Leucaena* species, VAM colonization level was lowest in *L. retusa* and highest in *L. leucocephala* (Fig. 4). At the lowest concentration of soil solution P tested, VAM colonization levels were not different between *Sesbania* species (Fig. 5). At a soil solution P concentration of 0.02 mg/l, significant reduction in VAM colonization was noticed in *L. trichodes* and *S. pachycarpa*. At 0.2 mg P/l, VAM colonization was significantly reduced in all plant species. At this P concentration, VAM colonization levels of *L. diversifolia* and *L. leucocephala* were higher than those of other *Leucaena* and *Sesbania* species (Figs. 4, 5).

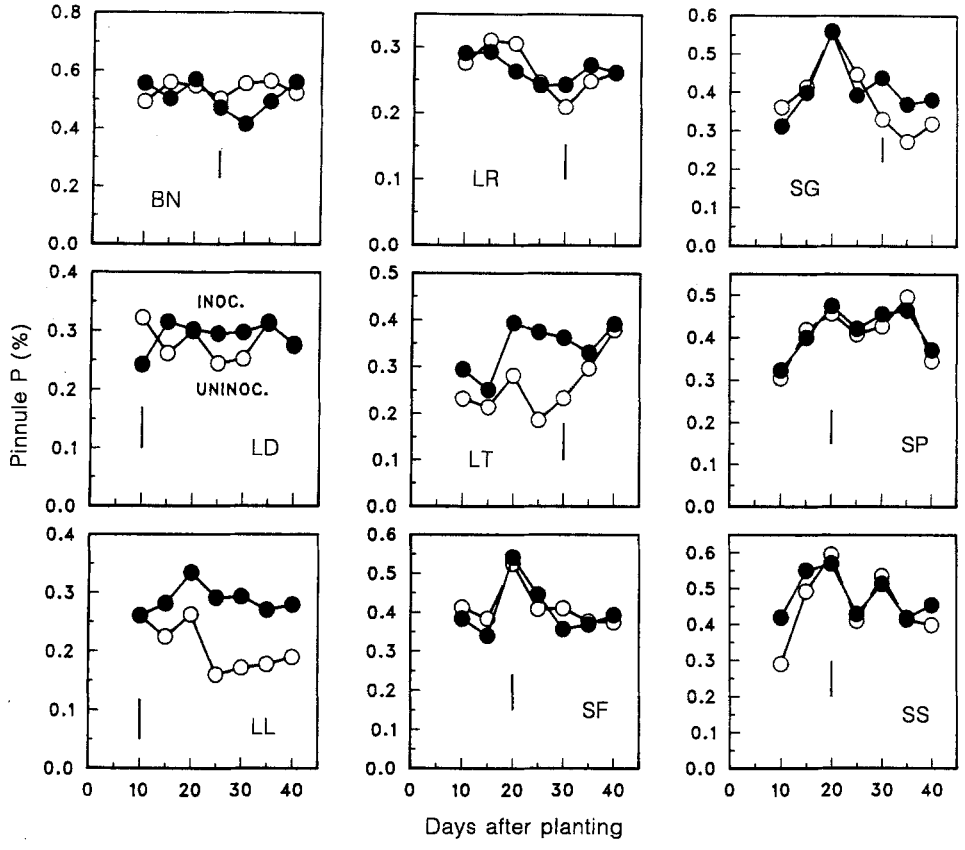

Fig. 3. P concentration in leaf disks of *B. nigra* and in pinnules of *Leucaena* and *Sesbania* species as influenced by inoculation with *G. aggregatum* at a soil solution P concentration of 0.2 mg/l. *Vertical bars* represent LSD values at the 5% level. *INOC.*, Inoculated with *G. aggregatum* (●); *UNINOC.*, uninoculated (○). Species abbreviations are the same as in Fig. 1

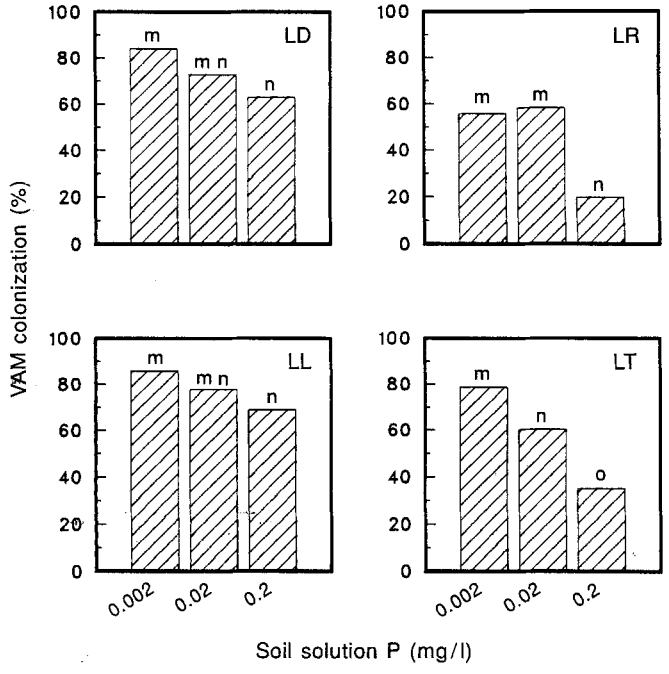

Fig. 4. Influence of soil solution P concentration on mycorrhizal colonization of roots in *Leucaena* species. Colonization data were subjected to angular transformation before analysis. Non-transformed means are presented. Means followed by the same letter within a species do not differ significantly at the 5% level. Species abbreviations are the same as in Fig. 1

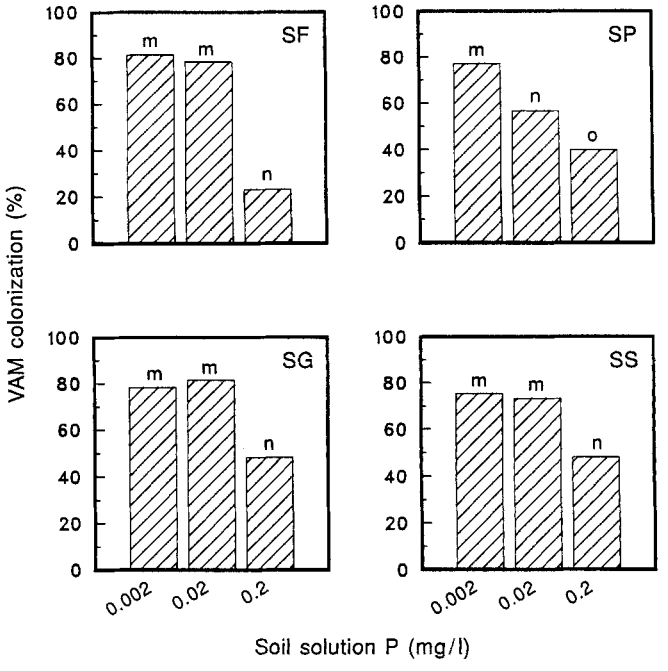

Fig. 5. Influence of soil solution P concentration on mycorrhizal colonization of roots in *Sesbania* species. Colonization data were subjected to angular transformation before analysis. Means followed by the same letter within a species do not differ significantly at the 5% level. Species abbreviations are the same as in Fig. 1

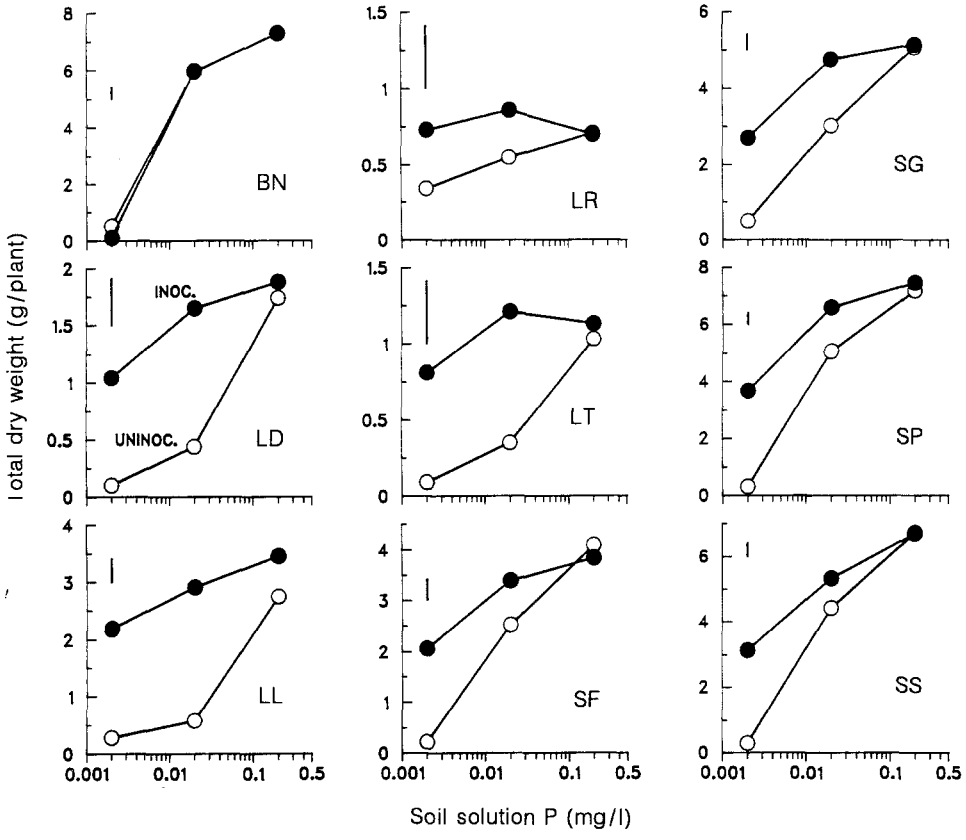

Fig. 6. Influence of soil solution P concentration and inoculation with *G. aggregatum* on total dry weight of *B. nigra* and species of *Leucaena* and *Sesbania*. *Vertical bars* represent LSD values at the 5% level. *INOC.*, Inoculated with *G. aggregatum* (●); *UNINOC.*, uninoculated (○). Species abbreviations are the same as in Fig. 1

Dry matter yield and nutrient concentration

Dry matter production in *B. nigra* was significantly stimulated by soil solution P concentration but not by mycorrhizal inoculation (Fig. 6). In the remaining plant species, except in *L. retusa*, total dry matter yield was significantly increased by mycorrhizal inoculation at soil solution P concentrations of 0.002 and 0.02 mg/l. At 0.02 mg P/l, the impact of inoculation on dry matter yield in *Leucaena* species was appreciably greater than in species of *Sesbania*.

Inoculation of soil with *G. aggregatum* significantly increased the P concentration of shoots and roots of all plant species except that of *B. nigra* at the lowest two concentrations of soil P (Figs. 7, 8). Mycorrhizal inoculation also significantly increased the P concentration of shoots and roots of all *Leucaena* species except that of *L. retusa* and of *S. grandiflora* at the highest concentration of soil solution P. At this concentration of P (0.2 mg/l), mycorrhizal inoculation did not influence the concentration of P in shoots and roots of *S. formosa*, *S. pachycarpa* or *S. sesban*. Except for *S. grandiflora*, the tissue P contents of *Sesbania* species changed to a higher degree in response to soil solution P increments than those of *Leucaena* species.

The K content of *B. nigra* increased with the first increment of soil solution P (Fig. 9). Further increase in P level had no effect on tissue K content. In most species of *Leucaena*, mycorrhizal inoculation increased tissue K content, the effect being most pronounced in *Leucaena leucocephala*, followed by *L. diversifolia*, *L. retusa* and *L. trichodes*. In *Sesbania* species, a positive K response to VAM infection was noted mostly at the initial soil solution P concentration and only in *S. formosa*, *S. grandiflora* and *S. sesban*.

Mycorrhizal inoculation significantly improved the tissue Cu concentrations of *L. diversifolia*, *L. leucocephala* and *L. trichodes* at all soil solution P concentration (Fig. 10). Mycorrhizal and nonmycorrhizal *L. retusa* did not differ in Cu content. The Cu contents of mycorrhizal and nonmycorrhizal *Sesbania* species, on the other hand, were only significantly different from each other at a soil solution P concentration of 0.002 mg/l. At higher P concentrations, tissue Cu content in these plants declined and differences between mycorrhizal and nonmycorrhizal plants disappeared. The nonmycorrhizal reference species *B. nigra* had the highest initial P concentration observed but the Cu content dropped markedly (more so than with any other species) as the P concentration was increased to 0.02 mg/l, and then less markedly from 0.02 to 0.2 mg/l. The negative effect of P on tissue Cu was most pronounced in mycorrhizal than in nonmycorrhizal plants and appears to be inversely related to the degree to which test species responded to inoculation with *G. aggregatum*.

Mycorrhizal inoculation and soil solution P concentrations generally resulted in patterns of Zn accumulation that were similar to those observed for Cu, except that the effects of the treatments on Zn content were relatively less pronounced compared to their effect on Cu content (Fig. 11).

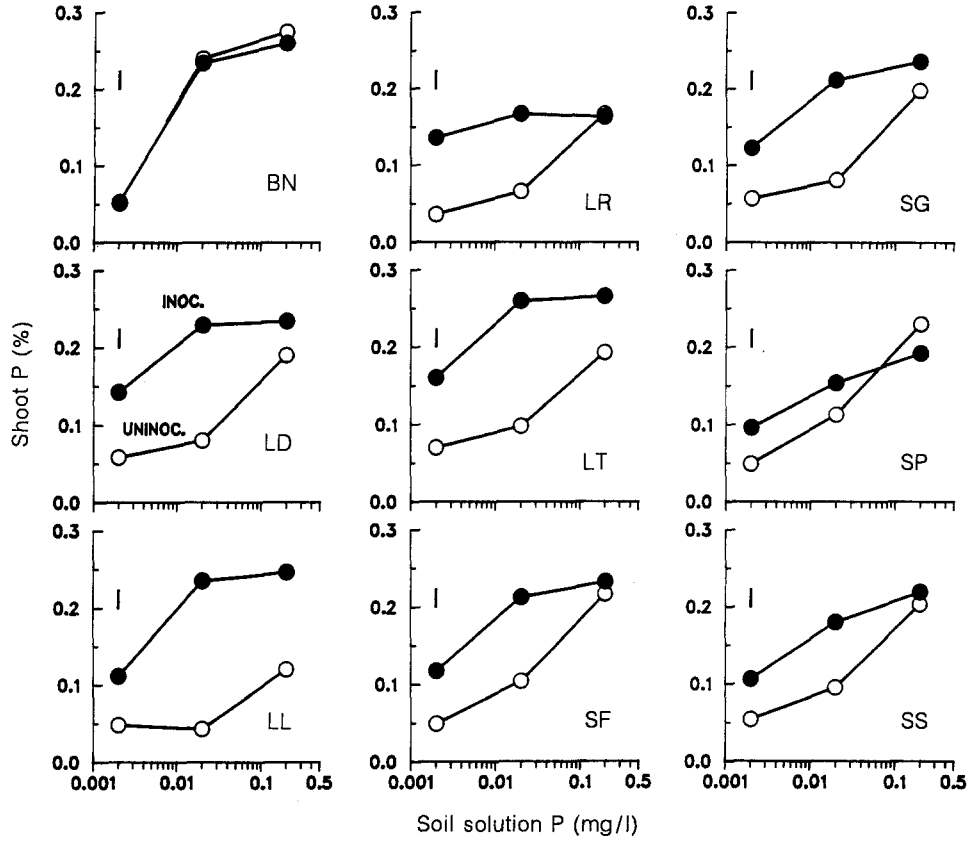

Fig. 7. Influence of soil solution P concentration and inoculation with *G. aggregatum* on P concentration in shoots of *B. nigra* and species of *Leucaena* and *Sesbania*. *Vertical bars* represent LSD values at the 5% level. *INOC.*, Inoculated with *G. aggregatum* (●); *UNINOC.*, uninoculated (○). Species abbreviations are the same as in Fig. 1

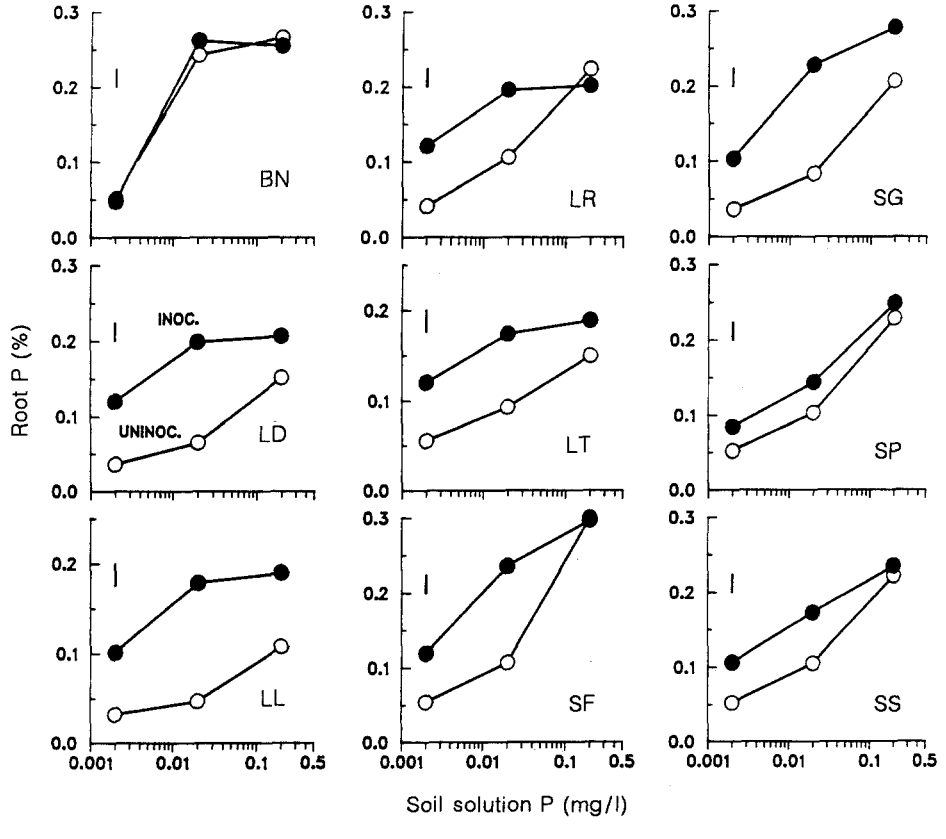

Fig. 8. Influence of soil solution P concentration and inoculation with *G. aggregatum* on P concentration in roots of *B. nigra* and species of *Leucaena* and *Sesbania*. *Vertical bars* represent LSD values at the 5% level. *INOC.*, Inoculated with *G. aggregatum* (●); *UNINOC.*, uninoculated (○). Species abbreviations are the same as in Fig. 1

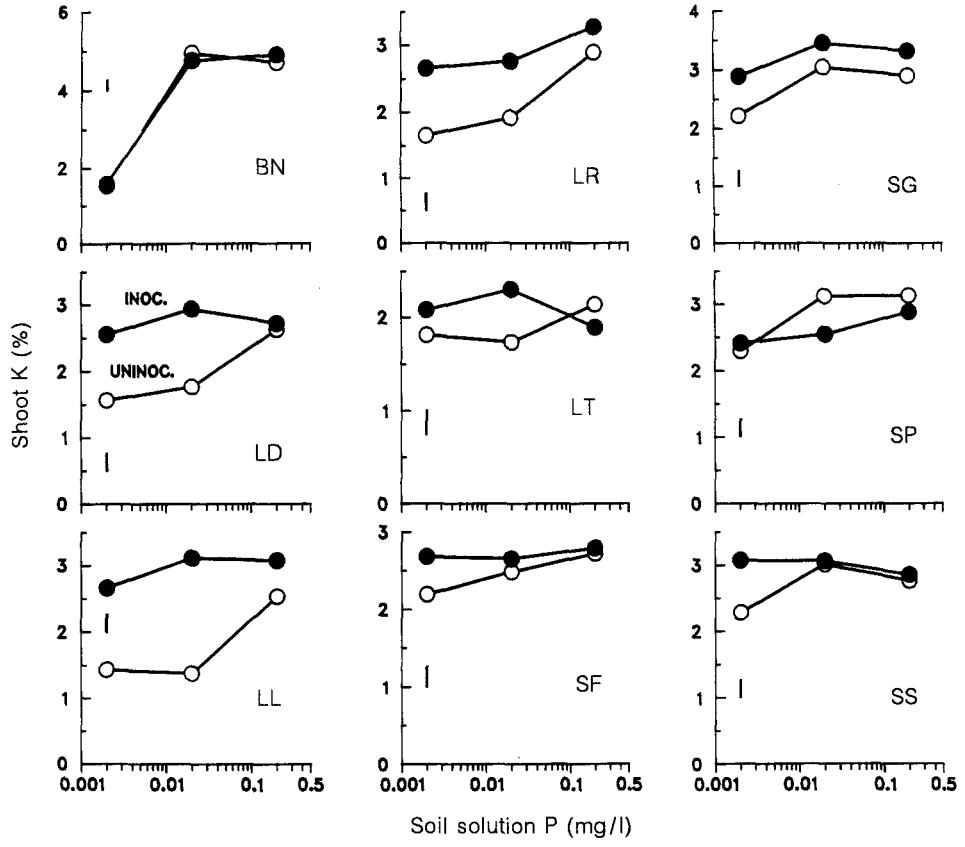

Fig. 9. Influence of soil solution P concentration and inoculation with *G. aggregatum* on K concentration in shoots of *B. nigra* and species of *Leucaena* and *Sesbania*. *Vertical bars* represent LSD values at the 5% level. *INOC.*, Inoculated with *G. aggregatum* (●); *UNINOC.*, uninoculated (○). Species abbreviations are the same as in Fig. 1

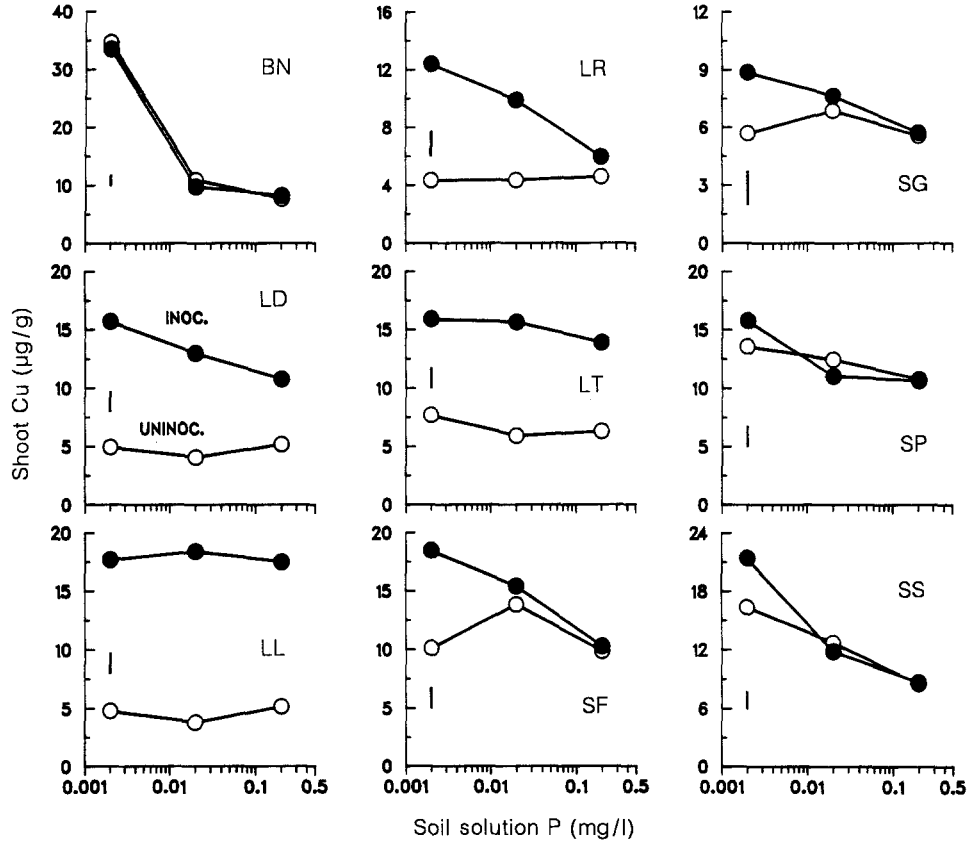

Fig. 10. Influence of soil solution P concentration and inoculation with *G. aggregatum* on Cu concentration in shoots of *B. nigra* and species of *Leucaena* and *Sesbania*. *Vertical bars* represent LSD values at the 5% level. *INOC.*, Inoculated with *G. aggregatum* (●); *UNINOC.*, uninoculated (○). Species abbreviations are the same as in Fig. 1

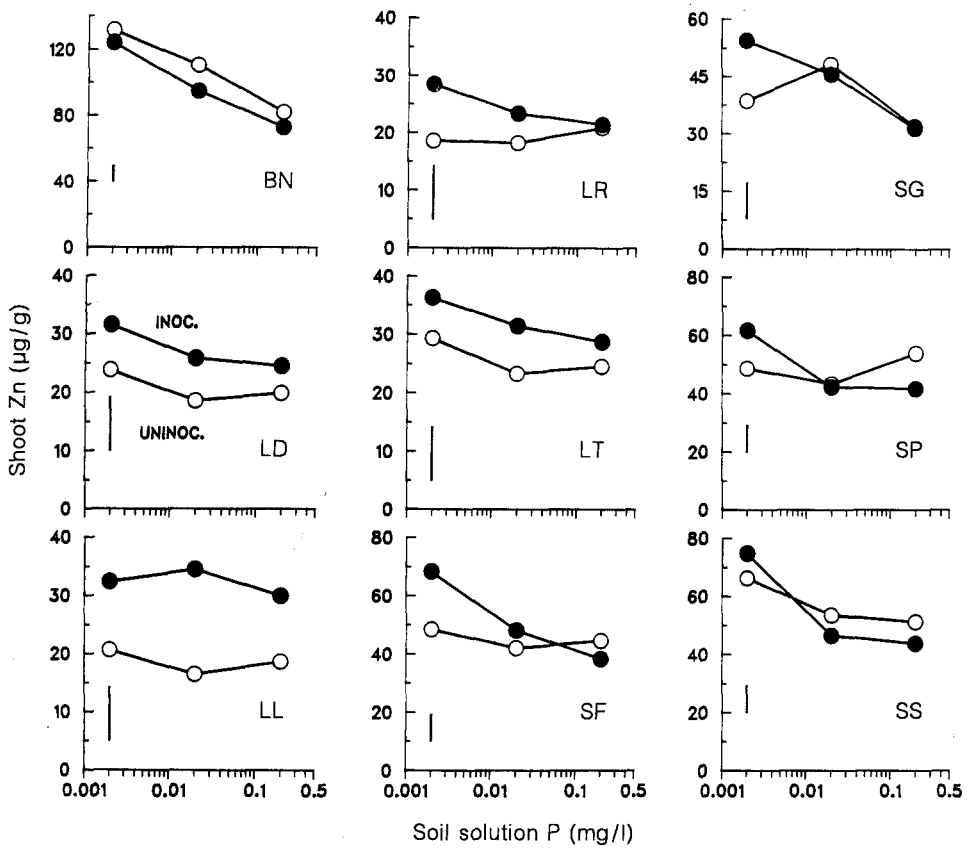

Fig. 11. Influence of soil solution P and inoculation with *G. aggregatum* on Zn concentration in shoots of *B. nigra* and species of *Leucaena* and *Sesbania*. *Vertical bars* represent LSD values at the 5% level. *INOC.*, Inoculated (●); *UNINOC.*, Uninoculated (○). Species abbreviations are the same as in Fig. 1

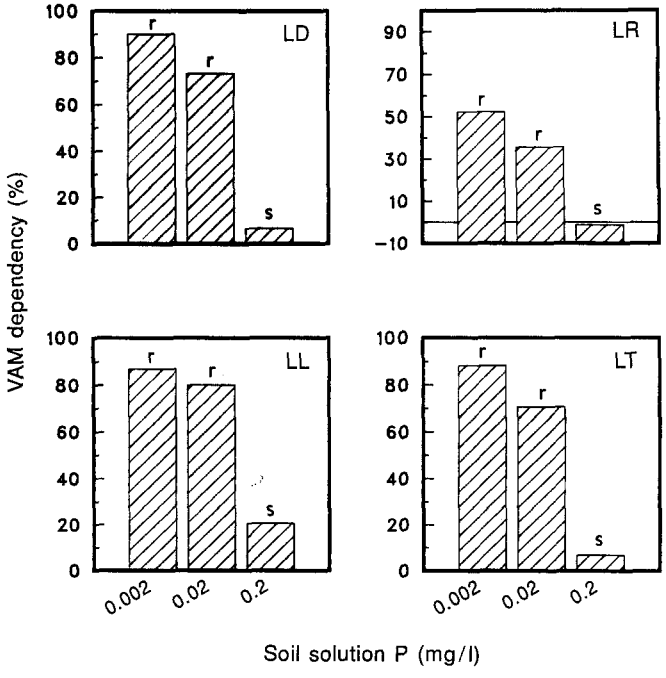

Fig. 12. Influence of soil solution concentration on mycorrhizal dependency of *Leucaena* species. Means followed by the same letter within a species do not differ significantly at the 5% level. Species abbreviations are the same as in Fig. 1

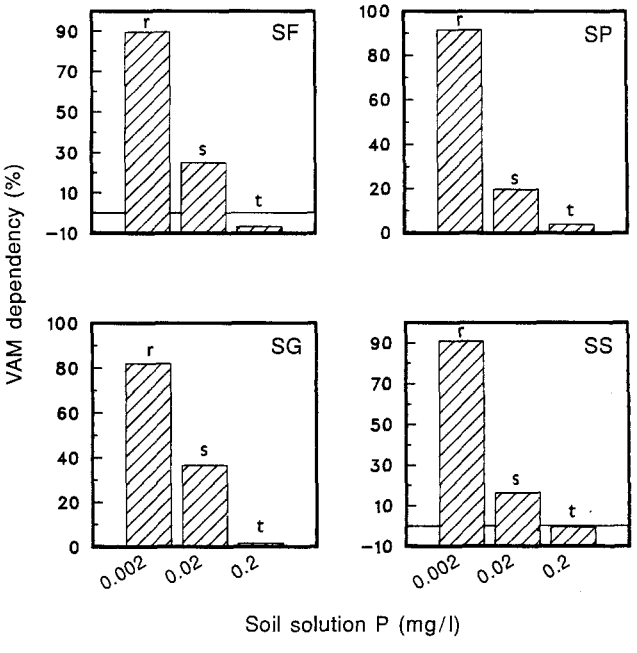

Fig. 13. Influence of soil solution P concentration on mycorrhizal dependency of *Sesbania* species. Means followed by the same letter within a species do not differ significantly at the 5% level. Species abbreviations are the same as in Fig. 1

At the lowest concentration of soil solution P, all the legume species tested except *L. retusa* had similar mycorrhizal dependency values (Figs. 12, 13). Species differences in mycorrhizal dependency became evident as soil solution P was increased.

The main effects of VAM inoculation, soil solution P concentrations and plant species on dry matter yield and concentrations of P, K, Cu and Zn were significant at the 5% level. The two factor interaction effects were also significant at the 5% level. The three factor interac-

tion effect was significant at the 5% level for all parameters except for shoot Zn concentration.

Discussion

Mycorrhizal dependency

Mycorrhizal dependency of all the species tested except that of *L. retusa* were similar at a soil solution P concentration of 0.002 mg/l. However, the species separated into distinct VAM dependency categories as soil P concentration was increased from 0.002 to 0.02 mg/l and 0.02 to 0.2 mg/l. Therefore, the critical P concentrations useful for differentiating host species into VAM-dependency categories are 0.02 and 0.2 mg/l. Based on these data, we have proposed five VAM-dependency categories as shown below:

1. Very highly dependent: species with an MD of 75% or higher at a soil solution P concentration of 0.02 mg/l and responding significantly to inoculation at 0.2 mg P/l. Example: *L. leucocephala.*

2. Highly dependent: species with an MD of 50–75% at a soil solution P concentration of 0.02 mg/l and not responding significantly at 0.2 mg/l. Examples: *L. diversifolia* and *L. trichodes.*

3. Moderately dependent: species with an MD of 25–50% at a soil solution P concentration of 0.02 mg/l. Examples: *L. retusa* and *S. grandiflora.*

4. Marginally dependent: species with an MD of less than 25% at a soil solution P concentration of 0.02 mg/l. Examples: *S. formosa*, *S. pachycarpa* and *S. sesban.*

5. Independent: species that are not colonized by VAM fungi or those that do not respond positively to VAM infection. Example: *B. nigra.*

Our results support the earlier hypothesis of Linderman and Hendrix (1982) who argued against the use of a single P concentration for differentiating "VAM-dependent" species from "VAM-responsive" ones. However, it is evident from our findings that the distinction between VAM responsiveness and VAM dependency is tenuous, since any symbiotically infected species is likely to be dependent on the fungi at some low soil solution P concentration at which the fungi can function normally.

Development of VAM effectiveness

Previously, Habte and Manjunath (1987) established that a target soil solution P concentration of 0.02 mg/l was optimal for VAM effectiveness in *L. leucocephala.* While this relationship did not change in the current study, even though an established rather than an initial target P concentration was used, symbiotic effectiveness did not peak at 0.02 mg P/l for all test species. For instance, the maximum difference in pinnule P content between VAM and non-VAM plants occurred at a soil solution P concentration of 0.002 mg/l in *L. retusa* and in all *Sesbania* species except in *S. grandiflora* (Fig. 1). This difference was maximal at a soil solution P concentration of 0.02 mg P/l in *L. leucocephala, L. trichodes* and *S. grandiflora*, with values for *L. diversifolia* being similar at both soil solution P concentrations. In general, therefore, maximum VAM effectiveness is likely to occur at soil solutions P concentrations lower than 0.02 mg/l in species that are marginally dependent on VAM fungi. By the same token, maximum VAM activity in highly and very highly VAM-dependent species appears to be associated with a soil solution P concentration of 0.02 mg/l. For the moderately VAM-dependent species, the P level associated with maximum VAM activity lies between 0.002 and 0.02 mg/l, since maximum VAM activity in two of our moderately-dependent species *L. retusa* and *S. grandiflora* occurred at 0.002 and 0.02 mg P/l, respectively. Matching VAM dependency of host species with soil solution P status is, therefore, a critical aspect of the effort that must be directed towards maximizing the beneficial effects of VAM fungi in agriculture.

Dry matter yield and nutrient concentration

The congruency between shoot and root P content of VAM-infected and uninfected plants and total biomass yields supports previous findings that the beneficial effects of VAM infection on host species is largely explainable in terms of improved P uptake (Hayman 1983; Pacovsky and Fuller 1986). Nevertheless, it is interesting to note that at the highest soil solution P concentration, increase in tissue P concentration due to VAM infection did not lead to increase in dry matter accumulation in most test species. Such unnecessary accumulation of P in the plant could contribute to the depletion of soil P.

Plants differing in their VAM dependency responded differently to soil solution P concentrations with respect to tissue Cu and Zn concentrations. In general, there was an inverse relationship between soil solution P and tissue Cu and Zn. Hence in the most VAM-dependent species, *L. leucocephala,* the effect of P on Cu and Zn content was minimal, while the most pronounced P-induced decline in the two elements was observed in our nonmycorrhizal reference species *B. nigra.* Since species exposed to similar soil solution P concentrations responded differently in this regard, soil P must have exerted its influence indirectly through its effect on tissue P. This is evident from the fact that marginally and moderately VAM-dependent species and *B. nigra* by and large had higher tissue P concentrations compared to the highly and very highly dependent species (see Figs. 1–3). The decrease in the concentrations of shoot Cu and Zn with increase in soil solution P concentrations may also have been caused by the dilution effect of growth (Lambert et al. 1979), since increases in soil P concentrations resulted in significant increases in dry matter. In species that were infected with *G. aggregatum*, P-induced reduction in tis-

sue Cu and Zn content may also reflect reduced uptake of the micronutrients because of the suppression of VAM activity at high P concentrations (see Fig. 4). It is well known that high soil P suppresses VAM activity mainly through its effect on the P concentration in plants (Bowen 1987; Menge et al. 1978).

It is clear from our data not only that VAM fungi improve the uptake of nutrients such as P, K, Cu, and Zn but also that plants having different degrees of dependence on the fungi differ in the pattern by which they accumulate these nutrients. Thus, in addition to its usefulness in the prediction of VAM inoculation effects, knowledge of VAM dependency of plant species is crucial to a better understanding of plant nutrition.

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