PN-ABK-109
ISN 74945

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COMMUN. SOIL SCI. PLANT ANAL., 22(13&14), 1423-1437 (1991)

RELATIONSHIP BETWEEN MYCORRHIZAL DEPENDENCY AND RATE VARIABLES ASSOCIATED WITH P UPTAKE, UTILIZATION AND GROWTH

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ABSTRACT: A greenhouse investigation was undertaken to determine the relationship between mycorrhizal dependency of host species and rate variables associated with their P uptake, P utilization and growth. The results indicated that species that are marginally dependent on VAM fungi tend to have lower P utilization efficiency, higher P absorption rate and higher growth rates compared to moderately or highly VAM dependent species when they are not colonized by mycorrhizal fungi. The results also demonstrated that species differing in growth rates could differ in their mycorrhizal dependency even when they have similar root morphological characteristics.

INTRODUCTION

Because the demand for P is directly related to growth rate of plant species (21,24), it is possible that growth rate may influence the extent to which a host plant depends on vesicular-arbuscular mycorrhizal (VAM) fungi. Failure of the woody plant species *Metrosidores umbellata* to respond to VAM colonization despite its coarse roots with few root hairs has been attributed to the plant's slow growth habit (7). Hayman (9) and Janos (17) also believe that growth rate may be an important physiological factor that determines mycorrhizal dependency (MD) of a plant species in addition to root morphology. Graham and Syvertsen (5) compared leaf area expansion rates of different citrus rootstocks and observed that high leaf area expansion rate was associated with greater degree of dependency of

Hawaii Institute of Tropical Agriculture and Human Resources Journal Series No. 3561.

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the rootstocks on VAM fungi. The relationship between MD and host growth rate is best elucidated by monitoring the growth rates of species having distinct mycorrhizal dependency. Recently, Habte and Manjunath examined the mycorrhizal dependency of selected species of *Leucaena* and *Sesbania* and identified five dependency categories (unpublished data).

The objective of this investigation was to determine the relationship between MD and rate variables associated with growth, P uptake (PUR) and P utilization (PUE) in a marginally VAM dependent species (*S. pachycarpa*), moderately VAM dependent hosts (*L. retusa* and *S. grandiflora*) or a very highly VAM dependent host (*L. leucoceohala*).

MATERIALS AND METHODS

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 (H_2O). A subsoil was selected because of low soil solution P level (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 adjusted to 6.0 by using $Ca(OH)_2$ (1.14 g/kg soil) to eliminate the potential adverse effect of Mn. Pots of 15x15 cm, 15x18 cm and 20x22 cm were filled with 2, 2.5 and 5 kg of soil (dry weight basis), respectively. A phosphorus sorption curve was used to determine the quantity of P needed to raise soil solution P level to 0.02 mg/L. (8). This is a level of P observed to be optimal for VAM activity in *Leucaena leucocephala* (6,16). Nitrogen was added as $Mg(NO_3)_2 \cdot 6H_2O$ at the rate of 34.6 mg N/kg soil. A nutrient solution containing KCl, $MgSO_4 \cdot 7HO$, $CuSO_4 \cdot 5H_2O$, H_3BO_3, $Na_2MoO_4 \cdot 2H_2O$, and $ZnSO_4 \cdot 7H_2O$ was added to soil at the rate of 100, 50, 5, 10, 0.5 and 10 mg/kg soil of K, Mg, Cu, B, Mo, and Zn, respectively. The pots containing soil were fumigated with 48 g of methyl bromide and 1.0 g of chloropicrin in a gas-tight chamber for 4 days at a time on two separate occasions (16). 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 of soils in some of the pots was carried out by mixing the contents of each pot with crude inoculum (25 g/kg soil) of *Glomus azggregatum* Schenck and Smith emend Koske consisting of sand, extramatrical spores, sporocarps, bits of hyphae and infected corn root segments. Uninoculated

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controls received sterilized sand (25 g/kg soil) and washings of crude inoculum after removal of *G. aggregatum* structures by filtration (Whatman No. 1 filter paper). Crude inoculum of *G. aggregatum* was prepared as described by Habte and Manjunath (6).

Seeds of *Leucaena leucoceohala* (Lam.) de Wit, *L. retusa* Benth., *Sesbania grandiflora* (L.) Poir. and *S. pachycarba* DC., Prodr. were pregerminated on water agar (0.9%) after scarification with conc. H_2SO_4. The scarification time for *L. leucocephala*, *L. retusa* and *S. grandiflora* was 20 min. Seeds of *S. pachycarba* were scarified for 40 min. *Brassica nigra* (L.) Koch. served as a nonmycorrhizal reference plant. Four pregerminated seeds were planted per pot. *Brassica 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 with sterile deionized water 6 times. After emergence, seedlings were thinned to two plants per pot.

There were 60 treatments resulting from a factorial combination of 2 levels of inoculation, 5 plant species and 6 times of harvest. Pots were arranged on greenhouse benches in a randomized complete block design with 3 replicates per treatment. Plants were maintained under greenhouse conditions and soil moisture was maintained at approximately 60% of water holding capacity.

Destructive sampling of plants was done every 7 days starting on the 10th day after planting (DAP) until the 45th day. Plants to be harvested on 10 and 17 DAP were grown in pots containing 2 kg of soil. Those to be harvested on 24 and 31 DAP were grown in pots containing 2.5 kg of soil while those to be harvested on 38 and 45 DAP were grown in pots containing 5 kg of soil. Dry weight and P content of shoots and roots and VAM colonization levels were measured. The proportion of roots colonized by VAM fungus was determined by the grid-line intersection method (4), after clearing roots with KOH and staining with acid fuchsin (0.15% in lactic acid and glycerol solution) (14). Phosphorus content in plant samples were determined by the molybdate blue method (20), after dry-ashing. Growth rate and PUR were calculated using the formulae proposed by Hunt (11). Phosphorus utilization efficiency is expressed as g of dry matter produced per mg of P taken up by the plant (2). Statistical analysis of data was done using a Statistical Analysis System (SAS) procedure (21).

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RESULTS AND DISCUSSION

Root samples of *Leucaena* and *Sesbania* species collected on 10 DAP were not colonized by VAM fungus (Fig. 1). At 17 DAP, the level of colonization was only 4% but increased rapidly thereafter. At final harvest, *L. leucocephala* and *S. grandiflora* were infected to a similar extent and had infection levels that were higher than those observed in roots of *L. retusa* and *S. pachycarba*. These results support the earlier observation of Janos (13) that MD of a plant species is not necessarily related to the extent of VAM colonization in roots.

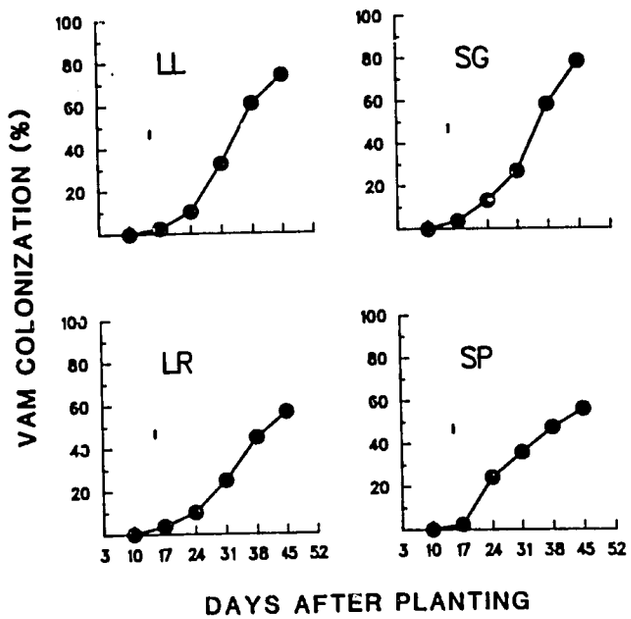

FIGURE 1. The development of vesicular-arbuscular mycorrhizal colonization in roots of *Leucaena* and *Sesbania* species. Vertical bars represent least significant difference (LSD) values at the 5% level. LL = *L. leucocephala*. LR = *L. retusa*. SG = *G. grandiflora*. SP = *S. pachycarpa*.

Inoculation of soil with *G. aggregatum* did not influence tissue P concentration, total P content and dry matter yield of *B. nigra* (Fig. 2 to 5). This is not surprising since Brassica nigra is not colonized by VAM fungi (10). Tissue P concentration of all plant species except that of *S. pachycarpa* decreased up to 24 DAP, which is probably caused by the dilution effect of growth, as dry matter usually accumulates faster than P uptake (2). However, tissue P concentrations

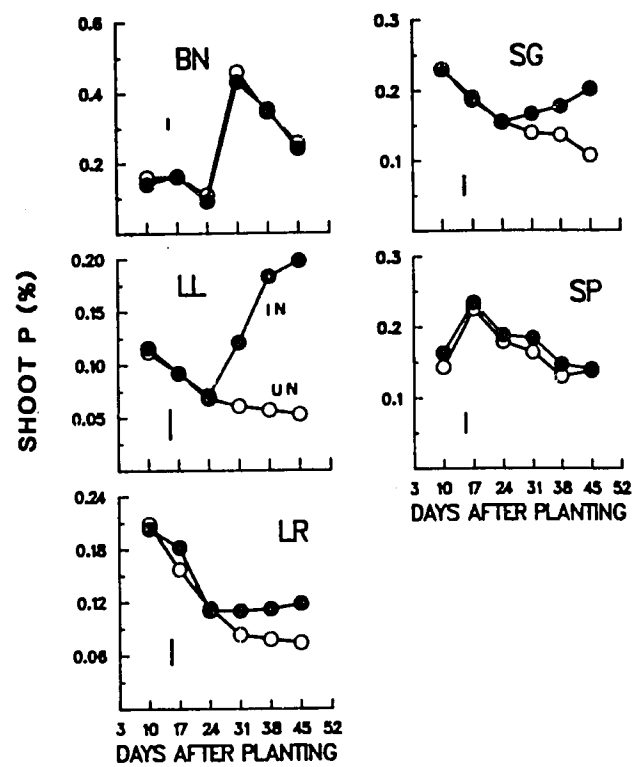

FIGURE 2. Phosphorus concentration in shoots of *B. nigra* and species of *Leucaena* and *Sesbania* grown in soil inoculated or uninoculated with *G. aggregatum*. Vertical bars represent LSD values at the 5% level. IN = Inoculated with *G. aggregatum*. UN = Uninoculated, BN = *B. nigra*. LL = *L. leucocephala*. LR = *L. retusa*. SG = *S. grandiflora*, and SP = *S. pachycarpa*

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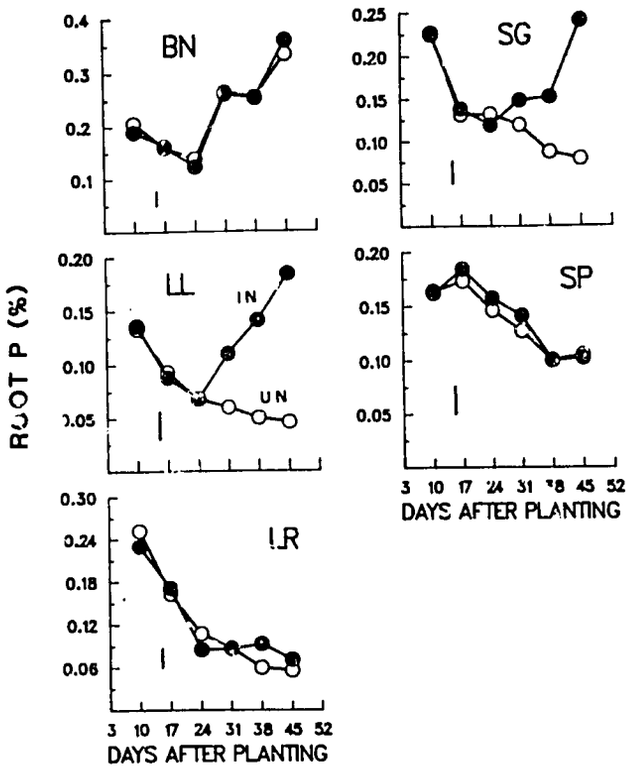

FIGURE 3. Phospohorus concentration in roots of *B. nigra* and species of *Leucaena* and *Sesbania* grown in soil inoculated or uninoculated with *G. aggregatum*. Vertical bars represent LSD values at the 5% level. IN = Inoculated with *G. aggregatum*, UN = Uninoculated BN = *B. nigra*, LL = *L. leucocephala*, LR = *L. retusa*, SG = *S. grandiflora* and SP = *S. pachycarpa*.

and total P contents of mycorrhizal *L. leucocephala* were higher than those of nonmycorrhizal counterparts on 31 DAP (Fig. 2, 3, and 4). Significant increases in dry matter yield due to VAM inoculation was observed in *L. leucocephala* on 45 DAP (Fig. 5). In general, tissue P concentrations, total P contents and dry matter yields of mycorrhizal *L. retusa* were not different from those of nonmycorrhizal

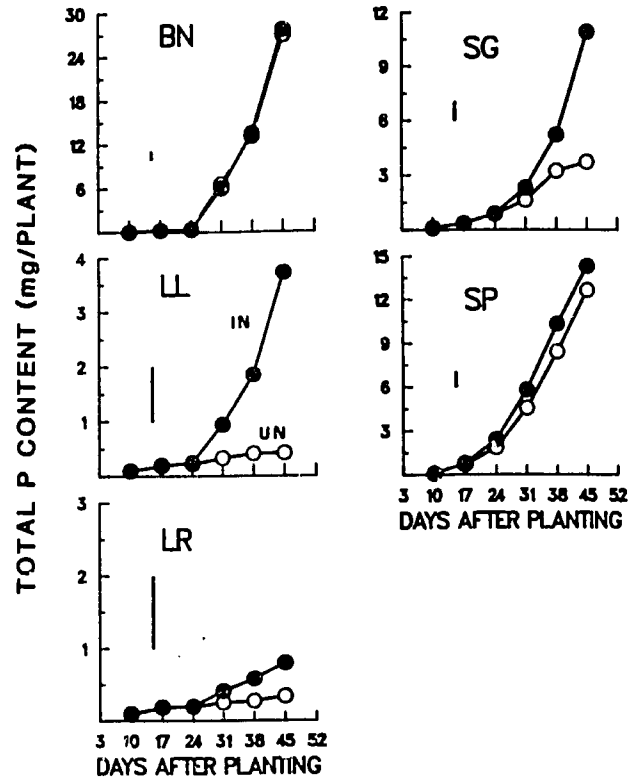

FIGURE 4. Total P content of *B. nigra* and species of *Leucaena* and *Sesbania* grown in soil inoculated with *G. aggregatum*. Vertical bars represent LSD values at the 5% level. IN = Inoculated with *G. aggregatum*, UN = Uninoculated, BN = *B. nigra*, LL = *L. leucocephala*, LR = *L. retusa*, SG = *S. grandiflora*, and SP = *S. pachycarpa*.

plants at all sampling intervals. While mycorrhizal inoculation significantly increased tissue P concentrations in *S. grandiflora* beginning on 31 DAP, it did not influence tissue P concentrations in *S. pachycarpa* at any sampling interval (Fig. 2 and 3). Mycorrhizal inoculation significantly increased total P content and dry matter yield of *S. grandiflora* and *S. pachycarpa* on 38 and 45 DAP,

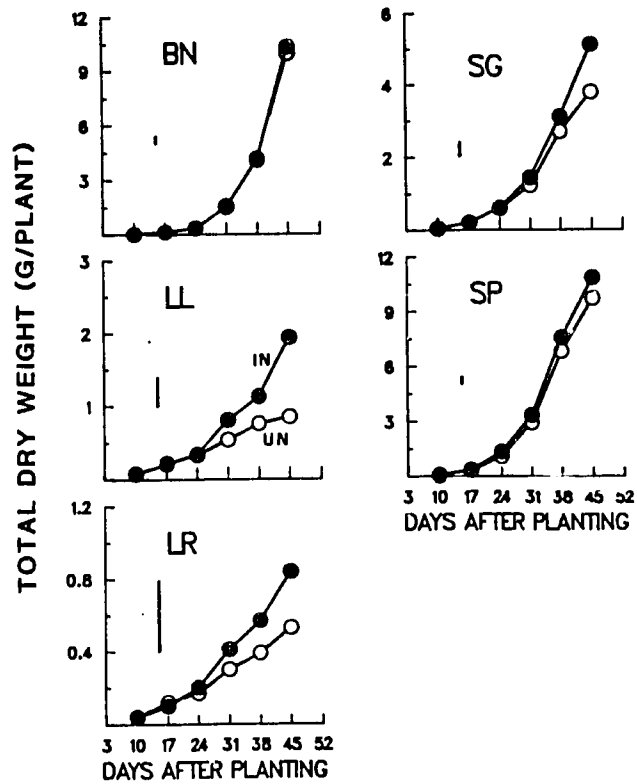

FIGURE 5. Total dry weight of *B. nigra* and species of *Leucaena* and *Sesbania* grown in soil inoculated or uninoculated with *G. aggregatum*. Vertical bars represent LSD values at the 5% level. IN = Inoculated with *G. aggregatum*, UN = Uninoculated, BN = *B. nigra*, LL = *L. leucocephala*, LR = *L. retusa*, SG = *S. grandiflora* and SP = *S. pachycarpa*.

respectively (Fig. 4 and 5). Tissue P concentrations and total P contents of non-mycorrhizal *Sesbania* species and *B. nigra* were higher than those of non-mycorrhizal *Leucaena* species at all times of sampling beginning on 24 DAP. This suggests that seedlings of plant species marginally dependent on VAM fungi are more efficient in P absorption than highly dependent ones in the absence of VAM fungi.

As expected, increases in tissue P concentration and total P content due to VAM infection closely paralleled increases in dry matter yield. There was a delay in the onset of VAM activity in this study compared to what we observed in our previous study (17). We suspect that the delay was caused by a low density of infective propagules in the VAM inoculum used in the current study.

Phosphorus uptake rates of *B. nigra*, *L. retusa*, and *S. pachycarpa* were not influenced by inoculation of soil with *G. aggregatum* (Fig. 6). Phosphorus uptake

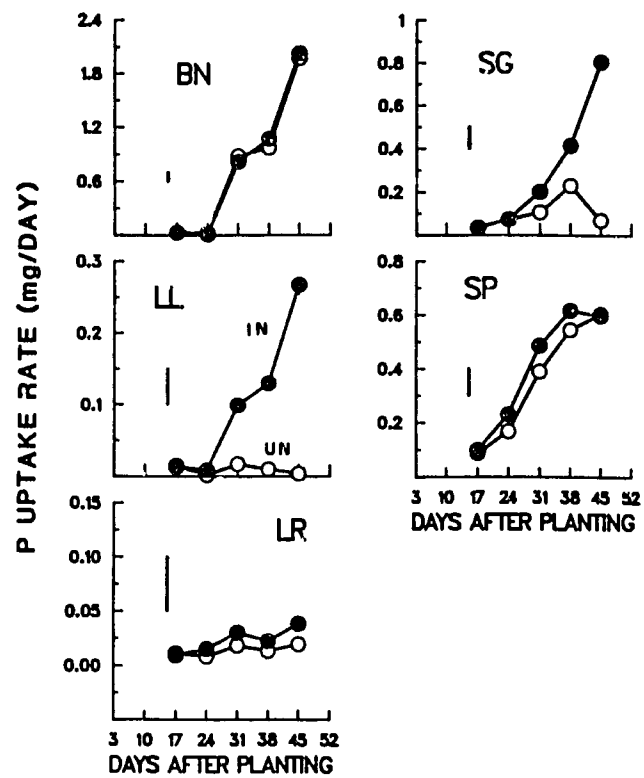

FIGURE 6. Phosphorus uptake rate (PUR) of *B. nigra* and species of *Leucaena* and *Sesbania* grown in soil inoculated or uninoculated with *G. aggregatum*. Vertical bars represent LSD values at the 5% level. IN = Inoculated with *G. aggregatum*, UN = Uninoculated BN = *B. nigra*, LL = *L. leucocephala*, LR = *L. retusa*, SG = *S. grandiflora*, and SP = *S. pachycarpa*.

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rates of Mycorrhizal *L. leucocephala* and *S. grandiflora* were higher than those of respective nonmycorrhizal plants beginning on 24 and 31 DAP, respectively, a phenomenon attributable to the development of external hyphae which increased the absorptive surface of roots (13). In general, PUR values of nonmycorrhizal *Sesbania* species were higher than those of nonmycorrhizal *Leucaena* species at all sampling times. Of the species tested, *B. nigra* had the highest rate of P uptake. High PUR of *B. nigra* and *Sesbania* species appears to be related to their root morphology. *Brassica nigra* and *Sesbania* species were observed to have fine root system with numerous root hairs (19). Plant species with fine root system are known to be more efficient in absorbing diffusion-limited nutrients such as P, than those with coarse root system (1,3). This fact is further made clear by comparing PUR of *S. grandiflora* with that of *S. pachycarpa*. Mycorrhizal colonization significantly influenced PUR of *S. grandiflora* beginning on 31 DAP, whereas it had no effect on PUR of *S. pachycarpa*. *S. grandiflora* has restricted root length and root surface area compared to *S. pachycarpa* (19). These results clearly demonstrate the inverse relationship between MD and PUR and the direct relationship between root morphology and PUR. Data on PUE indicate that nonmycorrhizal *Leucaena* species utilized P for dry matter production more efficiently than nonmycorrhizal *Sesbania* species (Fig.7). In all plant species except in *S. pachycarpa*. PUE increased up to 24 DAP. Mycorrhizal inoculation significantly reduced PUE of *Leucaena* species and *S. grandiflora* after 24 days of planting whereas it did not influence PUE of *S. pachycarpa* at any sampling time. Significant reduction in PUE of *B. nigra*, mycorrhizal *L. leucocephala*. mycorrhizal *L. retusa* and mycorrhizal *S. grandiflora* appears to be related to increased PUR, which in turn increased total P content. In an earlier study, *L. leucocephala* was observed to have lower PUE and lower phosphorus utilization rate when it was mycorrhizal than when it was not (18). Phosphorus application has also been observed to reduce PUE of several plant species (15,23,24). Phosphorus utilization efficiency values of nonmycorrhizal *Leucaena* species were higher than those of nonmycorrhizal *Sesbania* species at all sampling times beginning on 17 DAP. Therefore, high PUE under nonmycorrhizal conditions appears to be a characteristic of plant species highly or moderately dependent on VAM fungi.

Growth rates of mycorrhizal *L. leucocephala* and mycorrhizal *S. grandiflora* were higher than those of their nonmycorrhizal counterparts at the 5th sampling

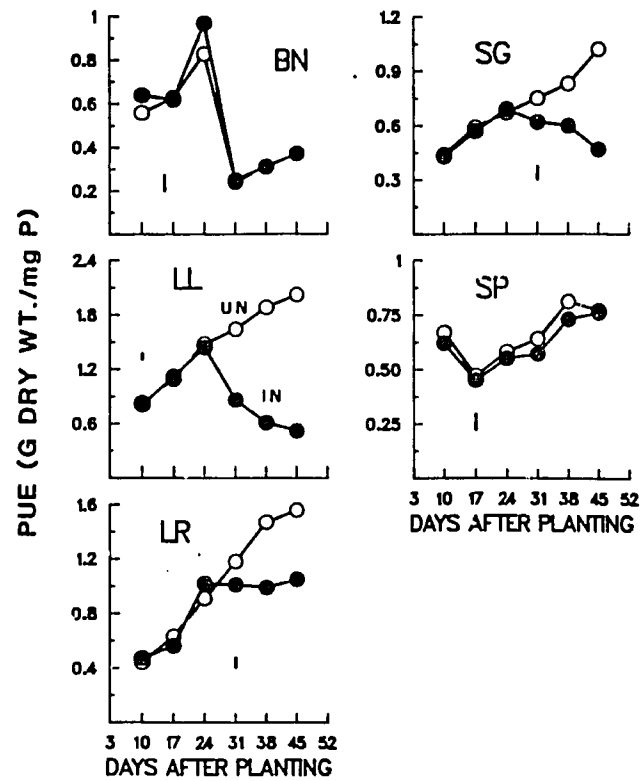

FIGURE 7. Phospohorus utilization efficiency (PUE) of *B. nigra* and species of *Leucaena* and *Sesbania* grown in soil inoculated with G. aggregatum. Vertical bars represent LSD values at the 5% level. IN = Inoculated with *G. aggregatum*, UN = Uninoculated, BN = *B. nigra*, LL = *L. leucocephala*, LR = *L. retusa*, SG = *S. grandiflora*, and SP = *S. pachycarpa*.

interval (Fig. 8). Growth rates of *B. nigra* and nonmycorrhizal *S. pachycarpa* were higher than those of nonmycorrhizal *L. leucocephala* at all sampling intervals. These results indicate that high non-mycorrhizal growth rate is a trait of plant species that are marginally dependent on VAM fungi. These plant species have fine root systems with high root surface areas, which enable them to absorb

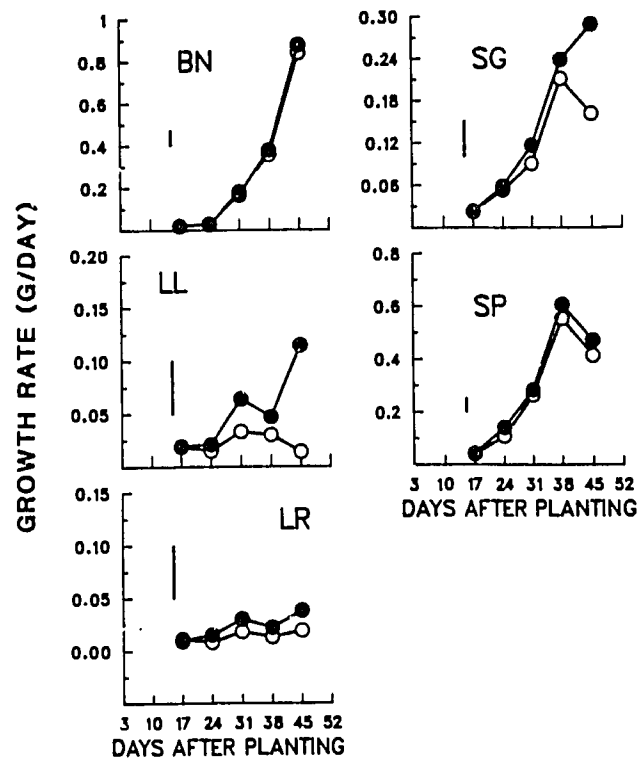

FIGURE 8. Growth rate of *B. nigra* and species of *Leucaena* and *Sesbania* grown in soil inoculated or uninoculated with *G. aggregatum*. Vertical bars represent LSD values at the 5% level. BN = *B. nigra*, LL = *L. leucocephala*, LR = *L. retusa*, SG = *S. grandiflora*, and SP = *S. pachycarpa*.

nutrients required for maintaining high growth rate. The growth rate of nonmycorrhizal *L. leucocephala* was not different from that of nonmycorrhizal *L. retusa*. However, once VAM fungus started facilitating P uptake (31 DAP), growth rate of mycorrhizal *L. leucocephala* was higher than that of mycorrhizal *L. retusa*, even though both species have similar root morphological characteristics (18). This indicates that growth rate of *L. leucocephala* is more sensitive to P supply than that of *L. retusa*. This is consistent with the tendency of mycorrhizal

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L. leucocephala to produce greater biomass than mycorrhizal *L. retusa* (Fig. 5). The lower VAM dependency of *L. retusa* relative to that of *L. leucocephala* suggests that species having similar root morphology can differ in their VAM dependency if their growth rates under mycorrhizal condition are different.

Our findings illustrate that plant species highly or moderately dependent on VAM fungi are characterized by low PUR, high PUE and low growth rate under nonmycorrhizal conditions. High PUE and low growth rate of plant species highly dependent on VAM fungi appear to be strategies for survival in nutrient-poor environments in the absence of VAM fungi. These traits favor the persistence of highly dependent plant species under conditions of nutrient inadequacy, which in turn may enhance the probability of colonization of the species by VAM fungi.

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