PN-ABK-471
ISN 75629

COMMUN. SOIL SCI. PLANT ANAL., 22(17&18), 1861-1872 (1991)

RESPONSE OF TWO SPECIES OF *CASSIA* AND *GLIRICIDIA SEPIUM* TO VESICULAR-ARBUSCULAR MYCORRHIZAL INFECTION[1]

M. Habte and D. Turk

Department of Agronomy and Soil Science, University of Hawaii, Honolulu, HI 96822

ABSTRACT: A pot experiment was conducted to evaluate the response to vesicular-arbuscular mycorrhizal (VAM) infection of two species of *Cassia* and *Gliricidia sepium* in an oxisol with two levels of soil P. At P level optimum for VAM activity, comparable levels of VAM infection were observed on the roots of the test species. However, the species reacted differently to VAM infection. Hence, VAM inoculation significantly stimulated dry matter accumulation in *C. spectabilis* and *G. sepium*, but had no positive influence on this variable in *C. reticulata*. These differences were not explainable in terms of tissue P status, because P uptake was significantly enhanced by inoculation in all the species tested. At the higher soil P level, roots of all species were colonized by the inoculum, but colonization level in *C. reticulata* and *G. sepium* was reduced. Nevertheless, only *C. spectabilis* benefitted from the presence VAM endophytes at this level of soil P. The results underline the significance of ascertaining the mycorrhizal requirements of host species before reliable VAM inoculation recommendation could be made.

INTRODUCTION

There has recently been a renewed interest in the development of sustainable agricultural systems in which the use of fertilizers and other chemical inputs are minum

1. Hawai Institute of Tropical Agriculture and Human Resources Journal Series No. 3572.

Copyright © 1991 by Marcel Dekker, Inc.

minimized. Tree legumes could play an important role in the development of such systems and in reforestation schemes due to their ability to form symbiotic association with nitrogen-fixing bacteria known as rhizobia. Many tree legumes have also been shown to form symbiotic associations with soil fungi known as vesicular-arbuscular mycorrhizal (VAM) fungi (3,10,13,19). VAM fungi are most important in the absorption of relatively immobile nutrients from soil, particularly P (9). This tendency of tree legumes to form dual symbiosis with rhizobia and VAM fungi is particularly importent for their establishment and growth in soils with low nutrient status.

Among tree legumes, *Leucaena leucocephala* has received the most attention from agricultural developers and has been widely used in reforestation and agroforesrtry programs in developing countries. However, it does not grow well in acid soils of low nutrient status (15). Consequently, there is a need to replace it with other fast-growing species that are acid-tolerant. Among species that have been suggested as possible replacement for *L. leucocephala* are *Gliricidia sepium, C. spectabilis* and *C. reticulata* (18,1). These species are native to Central or South America.

The objective of the current study was to determine the extent to which these species benefit from the VAM symbiosis during the initial phase of their growth.

MATERIALS AND METHODS

The soil used in this study was a subsurface (15-30 cm) Wahiawa silty loam (Tropeptic Eutrustox) with a pH (in water) of 5.3. It was collected from the Poamoho Research Station, Hawaii Institute of Tropical Agriculture and Human Resources, Oahu, Hawaii. The soil was crushed to pass through a 2-mm pore size sieve. Two kg portions of the air-dried soil were transferred into 15-cm plastic pots. Soil pH was adjusted to 6.0 by thoroughly mixing the contents of each pot with 3.75 g portions of $Ca(OH)_2$ 13 days prior to planting. Phosphorus was initially added as KH_2PO_4 at the rate of 400 or 1400 mg/pot. These quantities are determined to yield target soil solution P levels of 0.02 and 0.2 ug P/ml respectively, based on P sorption isotherm (5). The lower level has been found to be optimal for VAM activity in *L. leucocephala* while the higher level is associated with 95% of maximum yield in many species (4,7). The two levels will be hereafter referred to as optimum P and high P, respectively. The soils in the pots

·2

were moistened to approximately 60% of field capacity and incubated under shade for 2 days prior to fumigation. Pots containing the soils were fumigated with a mixture of methyl bromide and chloropicrin in an air-tight fumigation chamber for three days. The fumigants were allowed to dissipate for ten days prior to planting.

VAM inoculation of soil was achieved by thoroughly mixing 30 g portions of a crude inoculum of *Glomus aggregatum* consisting of sand, spores, bits of hyphae and pieces of infected roots with the soils in pots immediately before planting.

Seeds of *G. sepium* were surface-sterilized by immersion in 2.6% sodium hypochlorite for 4 minutes followed by 6 rinses with sterile water. After seeds were soaked overnight in sterile water, they were germinated for three days in petri plates containing water agar (0.9 % agar). Seeds of *Cassia* species were scarified by nicking the seed coat with a knife prior to surface sterilization. Other preplanting treatments were the same as for *G. sepium*.

Two seedlings of *G. sepium* or *C. spectabilis*, or three seedlings of *C. reticulata* were planted per pot. Seedlings that failed to emerge were replaced two weeks after planting. However, because the replants failed to catch up with those planted initially, pots with replanted seedlings were treated as missing plots during statistical analysis of data.

Plants were grown in the greenhouse under natural light (21° 51' N and 156° 22' W) during October and November of 1989.

Plants were watered as needed to approximately 60 % of field capacity using de-ionized water. Weeds were removed as necessary. Treatments in the two-by-two factorial experiment (two levels of inoculation and two levels of soil P) were arranged on greenhouse benches in a randomized complete block design with three replicates per treatment. Nutrients other than P were added eleven days after planting (7). An additional dose of P was also applied at this time at the same concentrations as before, because soil P determination at this time indicated that the targeted soil P levels were not attained.

Mycorrhizal effectiveness status in *C. reticulata* and *G. sepium* was monitored by employing the leaf disk technique of Aziz and Habte (2). Because *C. spectabilis* leaflets were very narrow they were not suited for leaf disk sampling; VAM effectiveness in this species was determined by monitoring P status of leaflet tips. For this purpose, leaflet tips that were approximately 10.0 mm long were

sampled. Leaf disk samples were obtained 24, 31, 38, and 45 days after planting while leaflet tip samples for *C. spectabilis* were collected on the latter 3 sampling dates.

Plants were harvested 45 days after planting. Roots were removed by gently shaking them from soil. They were washed in water and dried to constant weight at 70°C following removal of 0.5 gram portion samples (fresh weight) for mycorrhizal infection determination. Root samples were cleared using the method of Phillips and Hayman (13) and stained by employing a modification of the technique of Kormanik et al. (12). The percentage of infection was assessed using the method of Giovannetti and Mosse (6). Shoots were dried to constant weight at 70°C. Phosphorus content of leaflet/leaf disks, leaflet tips, and shoot samples were determined colorimetrically according to the molybdenum blue technique (14), following dry ashing at 500°C for 2-5 hours.

RESULTS

Despite the fact that the fumigants failed to kill weed seeds, none of the plants grown in the uninoculated soils exhibited evidence of VAM infection (Tables 1-3). The roots of plants grown in the inoculated soil were highly infected by the VAM fungus applied, although in the cases of *C. reticulata* and *G. sepium* high P level depressed VAM colonization. In *C. spectabilis* and in *G. sepium*,. dry matter yield was significantly increased by inoculation in the soil with P level optimum for VAM activity. At the higher P level, dry matter yield was significantly increased by inoculation in *C. spectabilis* (Table 1) but not in *C. sepium* (Table 2). However, the extent to which shoot dry matter yield responded to VAM inoculation in the former legume at the high soil P level was substantially lower than that observed at the optimum soil P level. VAM inoculation had no positive influence on shoot dry matter yield in *C. reticulata* at neither solution P level (Table 3). Root dry matter yield in *C. spectabilis* and *G. sepium* was stimulated by VAM inoculation in the soil with P level optimum for VAM activity but the influence of inoculation at the higher P level was not significant (Tables 1 and 2). By contrast, root dry matter yield of *C. reticulata* grown in the inoculated soils was half as much as those of plants grown in the uninoculated soils irrespective of soil P level (Table 3).

TABLE 1

Influence of Soil P Concentration and Mycorrhizal Inoculation on Dry Matter Accumulation, Shoot P Status, Shoot/Root Ratio and Mycorrhizal Colonization of Roots of Cassia spectabilis.*

Response Variable	Optimum P		High P	
	Uninoc.	Inoc.	Uninoc.	Inoc.
Root Dry wt(g)	0.09b	0.25a	0.32a	0.24a
Shoot Dry wt(g)	0.17c	1.05a	0.58b	0.97a
Shoot/Root Ratio	1.81b	4.16a	1.80b	3.95a
Infection (%)	0	77	0	80
Shoot P (%)	0.093b	0.329a	0.124b	0.335a

* Means followed by the same letter within a row are not significantly different from each other at the 5% level.

Mycorrhizal dependency calculated according to the formula of Plenchette e al. (17) showed that *C. spectabilis* was the most dependent of the species teste followed by *G. sepium*. *C. reticulata* exhibited no evidence of dependency on th VAM symbiosis. In fact, its dependency was calculated to be negative (-7.35 a the optimum soil P level and -12.6 at the higher P level).

Inoculation of soil with *G. aggregatum* led to increases in the shoot/root rati of all species at all P levels (Table 1-3). The increase in shoot/root ratio due t inoculation was greatest for *C. spectabilis* followed by *C. reticulata* and *G sepium*. In the case of *C. spectabilis* and *G. sepium*, the increase was primaril due to increases in shoot growth whereas in *C. reticulata* the increase was largel due to a decrease in root dry weigh.

5

TABLE 2

Influence of Soil P Concentration and Mycorrhizal Inoculation on Dry Matter Accumulation, Shoot P Status, Shoot/Root Ratio and Mycorrhizal Colonization of Roots of Gliricidia sepium.*

Response Variable	Optimum P		High P	
	Uninoc.	Inoc.	Uninoc.	Inoc.
Root Dry wt(g)	0.61b	0.80ab	1.41a	0.86ab
Shoot Dry wt(g)	0.77b	1.59a	1.73a	1.61a
Shoot/Root Ratio	1.25b	1.98a	1.51b	1.88a
Infection (%)	0	90	0	76
Shoot P (%)	0.079c	0.326b	0.110c	0.469a

* Means followed by the same letter within a row are not significantly different from each other at the 5% level.

Phosphorus status of leaflet/leaf disks and leaflet tips showed that P uptake was stimulated by VAM inoculation in all the species at both soil P levels tested (Fig. 1-3). The maximum P concentration attained ranged from 0.58 to 0.68 percent.

Shoot P content determined at the time of harvest revealed that plants had higher P status if grown in the presence of VAM inoculum rather than in its absence even at the high P level (Tables 1-3).

DISCUSSION

From the results given above, it is evident that *G. sepium* appears to be much less dependent on the VAM symbiosis than has been reported for *Leucaena*

TABLE 3

Influence of Soil P Concentration and Mycorrhizal Inoculation on Dry Matter Accumulation, Shoot P Status, Shoot/Root Ratio and Mycorrhizal Colonization of Roots of Cassia reticulata.*

Response Variable	Optimum P		High P	
	Uninoc.	Inoc.	Uninoc.	Inoc.
Root Dry wt(g)	0.48b	0.28c	0.85a	0.43bc
Shoot Dry wt(g)	0.84b	0.90b	1.50a	1.34a
Shoot/Root Ratio	1.87b	2.99a	1.78b	3.08a
Infection (%)	0	81	0	59
Shoot P (%)	0.132b	0.332a	0.196b	0.387a

* Means followed by the same letter within a row are not significantly different from each other at the 5% level.

leucocephala (7). The dependency indices we calculated clearly indicate that *C. spectabilis* is more dependent on VAM for growth than *G. sepium* at the low and high P levels used in this experiment. The tendency of *C. reticulata* not to benefit from VAM infection despite the fact that its roots were highly infected with the VAM fungus used and despite the fact that infection was associated with improved P uptake is puzzling. The negative dependency values calculated for it suggest the relationship prevailing between this species and the VAM species used was parasitic. This may reflect the higher cost in terms of carbohydrate allocation to the endophyte associated with the maintenance of symbiotic association between the VAM fungus and *C. reticulata*. The more than 50% reduction in root mass associated with the presence of VAM infection in this host lends support to this hypothesis. There was some evidence of depression of root mass by inoculation in

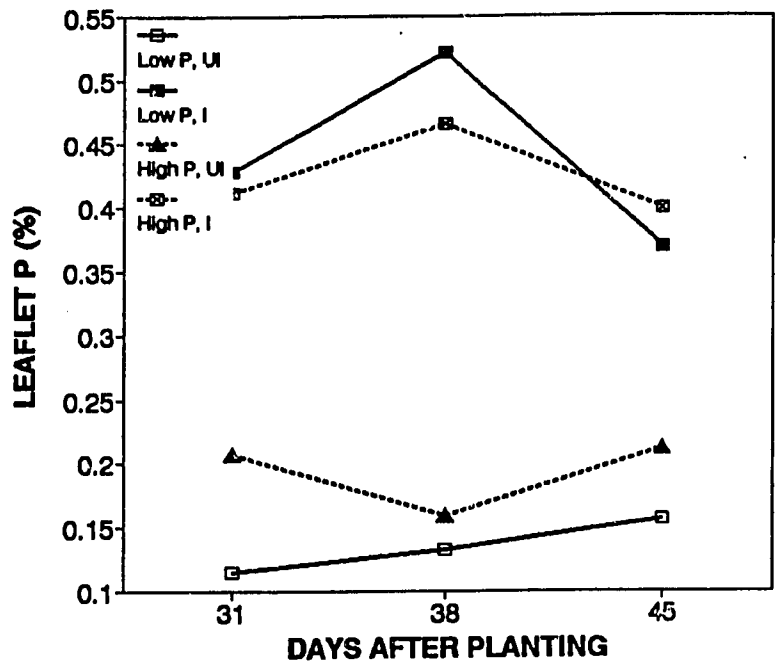

Figure 1. Influence of VAM inoculation and soil P on P status of leaflets of *C. spectabilis*. Inoculation effect was significant at both P levels on all sampling dates.

the other two species also. But the decreases were not significant and occurred only in plants grown at the high P level.

It is often believed that VAM fungi behave as parasites at very low soil P levels or at very high ones. The occurrence of parasitic interaction at soil solution P level near-optimum for VAM activity is hard to explain. To some extent, the lack of positive response to VAM inoculation of C. reticulata may have been caused by the ability of its roots to take up relatively high levels of P from soil. By the same token, the positive response to VAM infection of *C. spectabilis* at the high P level and the lack of a similar response in *G. sepium* suggest the greater efficiency of roots of the latter in absorbing P from soil in the absence of VAM fungi. The

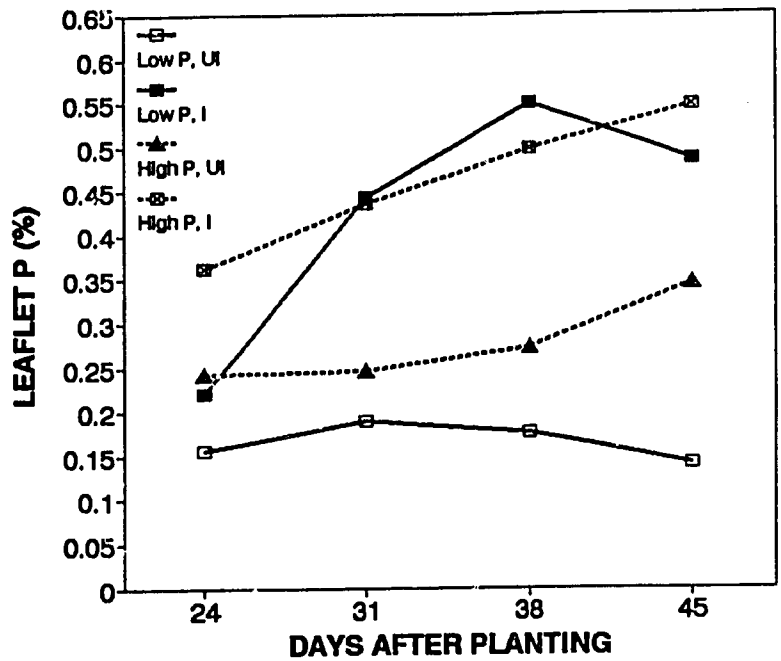

Figure 2. Influence of VAM inoculation and soil P on P status of leaf disks of G. *sepium*. Inoculation effect was significant at high P level on day 31 and at both P levels on days 38 and 45.

decline in VAM dependency with increase in soil solution P level observed in *G. sepium* and *C. spectabilis* is in agreement with the findings of Habte and Manjunath (7) who observed that mycorrhizal inoculation effect diminished progressively as soil P level was increased beyond a certain critical value.

Based on our results, it can be inferred that efficient P uptake due to symbiotic interaction with effective VAM fungi is a plausible explanation for the good growth of *C. spectabilis* and *G. sepium* often observed in poor soils. However, the superior performance of *C. reticulata* in acid infertile soils such as the ones found in Peru does not appear to be related to VAM actvity. It may be that *C. reticulata* forms efficient symbiosis with ectomycorrhizal fungi. Members of the

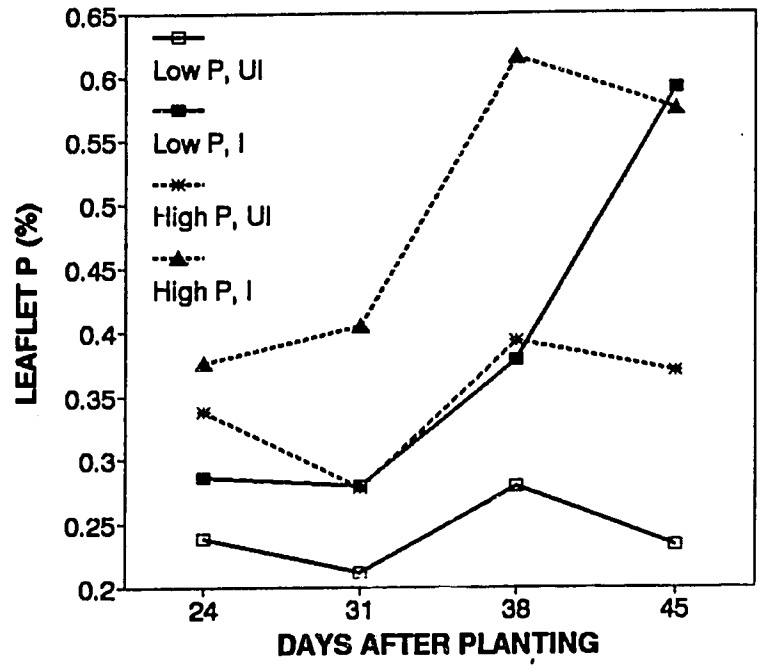

Figure 3. Influence of VAM inoculation and soil P on P status of leaflets of *C. reticulata*. Inoculation effect was significant at high P level on day 38 and at both P levels on day 45.

Caesalpinoideae, including species of *Cassia* from South America are known to form ectomycorrhizae (18).

Hogberg (8) has noted that the proportion of N fixing tress to non-fixing trees in Africa decreases as one goes from the low-rainfall arid zone into the tropical forest. Where few N fixing trees are present, N is unlikely to limit growth. Because P tends to be found at low solution levels in highly weathered soils of low pH, it may be more important for trees growing in such soils to have an efficient P-uptake mechanism than a mechanism for improved N nutrition.

Many of the arboreal species of the *Caesalpinoideae* subfamily of legumes do not fix N (1), yet they are among the dominant tree species over wide expanses of highly weathered soils (11). In soils where P is more limiting than N, mycorrhizal species with efficient P uptake such as *C. spectabilis* may have an advantage over other species, including those that fix nitrogen. The variation in the mycorrhizal requirements of the species examined strongly argues for clear definition of the VAM dependency of host species before effective VAM inoculation recommendations could be made.

ACKNOWLEDGMENT

This work was in part supported under a collaborative aggreement between IITA and the University of Hawaii.

REFERENCES:

1. Allen, O.N. and E.K. Allen. 1981. The Leguminosae: A Source Book of Characteristics, Uses, and Nodulation. University of Wisconsin Press, Madison, WI.

2. Aziz, T. and M. Habte. 1987. Determining vesicular-arbuscular mycorrhizal effectiveness by monitoring P status of leaf disks. Can. J. Microbiol. 33:1097-1101.

3. Borges, R.G. and W.R. Chaney. 1988. The response of *Acacia scleroxvla* Tuss. to mycorrhizal inoculation. Int. Tree Crops J. 5:191-201.

4. Fox, R.L. 1981. External phosphorus requirements of crops, pp. 223-239. IN: R. H. Dowdy, J.A. Ryan, V.V. Volk and D.E. Baker (eds.). Chemistry in the Soil Environment, American Society of Agronomy , Madison, WI.

5. Fox, R.L and E.J. Kamprath. 1970. Phosphate sorption isotherms for evaluating the phosphate requirements of soils. Soil Sci. Soc. Amer. Proc. 34:902-907.

6. Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84: 489-500.

7. Habte, M. and A. Manjunath. 1987. Soil solution phosphorus status and mycorrhizal dependency in *Leucaena leucocephala*. Appl. Environ. Microbiol. 53(4):797-801.

8. Habte, M., R.L. Fox, and R.S. Huang. 1987. Determining vesicular-arbuscular mycorrhizal effectiveness by monitoring P status of subleaflets of an indicator plant. Commun. Soil Sci. Plant Anal. 18(12):1403-1420.

9. Hayman, D.S. 1983. The physiology of vesicular-arbuscular mycorrhizal symbiosis. Can. J. Bot. 61:944-963.

10. Hogberg, P. 1986. Soil nutrient availability, root symbioses and tree species composition in tropical Africa: a review. J. Trop. Ecol. 2:359-372.

11. Hogberg, P. and G.D. Pierce. 1986. Mycorrhizas in Zambian trees in relation to host taxonomy, vegetation type and successional patterns. J. Ecol. 74: 775-785.

12. Kormanik, P.P., W.C Bryan and R.C. Schultz. 1980. Procedures and equipment for staining large numbers of plant roots for endomycorhizal assay. Can. J. Microbiol. 26:536-538.

13. Manjunath, A., D.J. Bagyaraj, and H.S. Gopala Gowda. 1984. Dual inoculation with VA mycorrhiza and Rhizobium is beneficial to Leucaena. Plant and Soil 78:445-448.

14. Murphy, J. and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27:31-36.

15. National Academy of Sciences. 1980. Firewood Crops: Shrub and Tree Species for Energy Production. National Academy of Sciences, Washington, DC.

16. Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:158-161.

17. Plenchette, C., J.A. Fortin and V. Furlan. 1983. Growth responses of several plant species to mycorrhizae in a soil of moderated P-fertility. Plant and Soil 70: 199-209.

18. Renier, M. 1946. La reforestation naturelle des savanes du Kwilu. Bull. Agric. Congo Belge 37(4):801-808.

19. Thomazini, L.I. 1974. Mycorrhiza in plants of the 'Cerrado'. Plant and Soil 41:707-711.

FEB 1992
RECEIVED
MANAGEMENT ENTITY
SOIL MANAGEMENT
CRSP