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Inoculation of *Vigna parkeri* with mycorrhizal fungi in an acid Florida spodosol

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Abstract

Vigna parkeri is a promising forage legume, but problems have been encountered with stand establishment and low tolerance to water stress. Vesicular-arbuscular mycorrhizal (VAM) fungi have been reported to ameliorate these problems. Our objectives were to (i) evaluate the effect of VAM inoculation on establishment and growth of *V. parkeri* in a spodosol under amended soil conditions, (ii) assess the effectiveness of introduced versus indigenous VAM fungi, (iii) compare VAM colonisation of *V. parkeri* grown alone with that of *V. parkeri* grown in combination with *Paspalum notatum*, and (iv) assess the effect of soil type and VAM isolate on colonisation of *V. parkeri*. Field and greenhouse studies were conducted in Pomona fine sand (sandy, siliceous, hyperthermic, Ultic Haplaquod) with low initial P status (Mehlich I-extractable P = 5 mg/kg) that was limed and amended with various rates of P. An attempt was made to equalise VAM inoculum densities in these studies, but the time required to conduct most-probable-number assays was sufficient for significant changes in propagule density to occur. The VAM fungi generally failed to adequately colonise *V. parkeri* in limed Pomona fine sand and shoot-P contents were found to be above the critical level for maximum biomass production in all treatments. Nonetheless, some differences were found in plant cover suggesting that selected stains of VAM fungi may be important in establishment. In the study where soil type was evaluated, colonisation was obtained even at shoot-P concentrations >5 g/kg, indicating that shoot-P concentration was not the only factor inhibiting colonisation in the Pomona soil. We suggest that pH may have also limited colonisation in these studies and that future work with this plant should include VAM isolates tolerant of acid soil environments.

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Resumen

Vigna parkeri *es un leguminosa forrajera prometedora, sin embargo se han encontrado problemas en su establecimiento y en su tolerancia a la escasez de agua. Se ha reportado que estos problemas pueden ser reducidos con el uso del hongo vesicular-arbuscular mycorrhizal (VAM). Nuestros objetivos fueron (i) evaluar el efecto del VAM en el establecimiento y crecimiento de* V. parkeri *en suelo tipo spodosol en condiciones mejoradas, (ii) evaluar la eficiencia del hongo VAM introducido con respecto al hongo nativo, (iii) comparar la colonización de VAM en un cultivo puro de* V. parkeri *y en un cultivo de* V. parkeri *asociado con* Paspalum notatum, *y (iv) evaluar el efecto del tipo de suelo y del VAM aislado sobre la colonización de* V. parkeri. *Los estudios fueron conducidos en invernadero y en el campo con suelo de arena fina Pomona (arenoso, con sílice, hipertérmico, Ultic Haplaquod) con un nivel inicial de P bajo (Mehlich I-P extraible = 5 mg/kg), al cual se le agregó cal y fue mejorado con varios niveles de P. Se intentó igualar las densidades del inóculo de VAM en estos estudios, pero el tiempo requerido para conducir el más adecuado número de ensayos fue suficiente para que ocurrieran cambios significativos en la densidad de los propágulos. En general el hongo VAM no colonizó adecuadamente* V. parkeri *en los suelos de arena fina Pomona tratados con cal y el contenido de P de los rebrotes fue arriba del nivel crítico para una máxima producción de biomasa en todos los tratamientos. Sin embargo, se encontraron algunas diferencias en la cobertura de las plantas, lo que sugiere que la selección de líneas del hongo VAM podría ser importante para el establecimiento. En el estudio en donde el tipo*

de suelo fue evaluado, la colonización se obtuvo aún en las concentraciones de P en los rebrotes > 5 g/kg, lo cual indica que la concentración de P en los rebrotes no es el único factor que inhibe la colonización en los suelos Pomona. Sugerimos que la colonización pudiera estar también limitada por el pH del suelo y por lo tanto los futuros trabajos con esta planta debieran incluir aislamientos de VAM tolerantes a suelos ácidos.

Introduction

Vigna parkeri is a promising forage legume for the humid subtropics (Pitman and Kretschmer 1984; Oram 1986; Pitman *et al.* 1988), although the legume's low tolerance to water stress (Oram 1986; Cook and Jones 1987) makes establishment difficult. The establishment of *V. parkeri* in spodosols of Florida has been particularly difficult due to low seedling vigour and a slow rate of spread (Pitman and Kretschmer 1984; Pitman and Singer 1985). The majority of native rangeland and improved pastures in central and south Florida are found on spodosols.

Root colonisation by vesicular-arbuscular mycorrhizal (VAM) fungi is often beneficial to host plants (Abbott and Robson 1984; Jeffries 1987). Mycorrhiza are thought to be especially important for legumes because of high plant demand for P (Mosse 1977a). Phosphorus is an important nutrient for legumes, not only for plant growth, but also for nodulation and N_2 fixation (Andrew and Robins 1969b; Bethlenfalvay and Yoder 1981). Little is known about the response of *V. parkeri* to VAM inoculation, although in preliminary studies with this legume VAM colonisation was found to be very low (O'Donnell *et al.* 1991).

Early work with VAM inoculation of tropical forage legumes demonstrated the importance of these fungi to legume growth. Crush (1974) found that VAM fungi enhanced both growth and nodulation of *Centrosema pubescens* and *Stylosanthes guianensis* in P-deficient soils. Mosse *et al.* (1976) found that VAM inoculation only improved growth of these 2 legumes when tissue-P concentrations were below 1.5 g/kg. Mosse (1977b) also demonstrated that inoculation with a VAM fungus improved the growth of *S. guianensis* in soils containing indigenous VAM fungi. Other researchers have studied VAM effects on *Pueraria phaseoloides* (Salinas *et al.* 1985) and *Macroptilium atropurpureum* (Lynd *et al.* 1985).

More recently, Medina *et al.* (1987; 1988a; 1988b; 1988c; 1990) conducted a series of experiments to assess responses of tropical forage legumes to VAM inoculation under amended soil conditions in a Florida spodosol. Inoculation with selected VAM fungi was found to improve growth of *M. atropurpureum, Aeschynomene americana, Stylosanthes hamata, S. guianensis* and *A. villosa* in a low-P soil (1 mg/kg Mehlich I-extractable P) (Medina *et al.* 1987). In a subsequent study in the same soil, *M. atropurpureum* and *A. americana* responded to VAM inoculation even with P applications of up to 40 mg/kg P (Medina *et al.* 1988c). Selected fungi were more effective than indigenous fungi in improving shoot growth under the amended soil conditions.

Roots of legumes are often colonised extensively by VAM fungi, although levels of colonisation can be quite variable (Strzemska 1975; Medina 1988a). Poor colonisation may be due to host-fungus incompatibility, low mycorrhizal dependency or suppression of colonisation by environmental factors. In general, there is a lack of host specificity in the VAM symbiosis (Mosse 1975; Gianinazzi-Pearson 1984; Harley 1985). Nonetheless, there is a wide range in the degree of host colonisation among VAM fungal isolates (Haas and Krikun 1985). Soil- and tissue-P levels, pH, light intensity, and temperature, among other factors, can affect VAM colonisation (Mosse *et al.* 1981). Furthermore, VAM colonisation of certain plants can be increased by growing them in combination with plants that are readily colonised by VAM fungi (Ocampo *et al.* 1980; Poole and Sylvia 1990).

The objectives of this research were to (i) evaluate the effect of VAM inoculation on establishment and growth of *V. parkeri* in a spodosol under amended soil conditions, (ii) assess the effectiveness of introduced *vs.* indigenous VAM fungi, (iii) compare VAM colonisation of *V. parkeri* grown alone with that of *V. parkeri* grown in combination with *Paspalum notatum*, and (iv) assess the effect of soil and VAM isolate on colonisation of *V. parkeri*.

Materials and methods

Studies were conducted in a virgin Pomona fine sand (sandy, siliceous, hyperthermic, Ultic Haplaquod), a typical spodosol found at the Agricultural Research and Education Center (AREC), Ona, Florida (27° 26'N latitude; 82° 55'W

longitude). Initial soil pH (2:1 H_2O:soil) of the Ap horizon (0–15 cm) was 4.4 and Mehlich I-extractable nutrient levels were: Ca, 170; Mg, 32; K, 26; P, 5; Mn, 44; Fe, 12; Zn, 8; and Cu, 0.08 mg/kg. Organic matter content of the soil was 26 g/kg.

Inocula preparation

Limed (4.5 t/ha of $CaCO_3$, pH 6.0) and pasteurised (8 h at 88 °C) Pomona fine sand was placed in 15-cm diam. plastic pots and inoculated with one of two isolates of *Glomus etunicatum* (isolate S-312 or S-329). *Paspalum notatum* was the host plant. At the end of 12 weeks of growth in the greenhouse, the soil and roots were removed from the pots, roots were cut into 1-cm lengths, and then remixed with the soil. An inoculum of native VAM fungi was prepared by collecting soil and roots from the Ap horizon of an established, unfertilised *P. notatum* pasture at the AREC-Ona; roots were removed from the soil, cut into 1-cm lengths, and then remixed with the soil. A portion of the limed, pasteurised soil was not inoculated with VAM fungi and was used for control treatments in the field and plant combination studies.

Most probable number (MPN) assay

Assays were conducted on the inocula approximately 6 weeks prior to each pot study in an attempt to adjust rates of application to equal inoculum densities, and again at the time of planting to determine actual densities when the studies were initiated. Ten-fold dilutions were used for S-312 and S-329, and 4-fold dilutions were used for the native inoculum. Limed (pH 5.5–6.0) and pasteurised Pomona fine sand was used as the diluent. Fifty grams (dry weight) of each dilution were placed into pinecell containers (63 ml, Ray Leach Conetainer Nursery, Canby, OR, USA). There were 5 dilutions in each assay and five replicates per dilution. *Zea mays* var. 'Early Sunglow' (W. Atlee Burpee and Co., Warminster, PA, USA) was used as the indicator plant. Plants were grown either in a greenhouse or growth chamber with mean daily maximum photosynthetic photon flux density (PPFD) ranging from 1000 to 1680 μmol/m^2/s. Plants were harvested 6 weeks after planting; roots were cleared with KOH and stained with trypan blue, and the entire root system inspected for VAM colonisation (Giovanetti and Mosse 1980).

VAM inoculation in amended Pomona soil

Field study. The experiment had a randomised complete-block design with a split-plot arrangement of treatments and 4 replicates per treatment. Main plots were VAM inoculation (*G. etunicatum* isolates S-312 and S-329, and control) and subplots were P application levels (25, 50 and 75 kg/ha). Main plots were 13.5 m by 3 m. Plots were limed with 4.5 t/ha of $CaCO_3$ 14 weeks prior to planting. Soil pH at the time of planting was 5.6, within the pH range (5.4–5.8) of optimum growth of *V. parkeri* in Pomona fine sand (O'Donnell *et al.* 1991). Mehlich I-extractable P was 5.0 mg/kg, which is considered very low according to the University of Florida standardised fertilisation recommendation for agronomic crops (Hanlon *et al.* 1990).

There were 6 furrows per plot; furrows were 4.5-m long and 5-cm deep with 40 cm between furrows. Each furrow received either 500 ml (approx. 475 g of dry weight) of inocula or pasteurised soil in the case of control plots. Inocula were covered to a depth of 3 cm with soil and scarified, rhizobia-inoculated ("cowpea" type) seed of *V. parkeri* were placed in the furrows and covered with an additional 2 cm of soil. Plots were seeded at the rate of 10 kg/ha. Treatment applications of P along with 166 kg/ha of K and 22 kg/ha of a micronutrient mix [TEM 300, Traylor Chemical and Supply Co., Orlando, Florida; elemental content (in g/kg): B, 24; Cu, 2.4; Fe, 144; Mn, 60; Zn, 56; and Mo, 0.6] were broadcast on the plots after plant emergence.

Establishment of *V. parkeri* was determined by stand ratings and estimates of plant cover. Plant stands were rated on a 0-10 scale based on plant growth and development, vigour, and colour; 0 indicated no plants present, 1 represented very small plants with few leaves and no branching or runners and a yellow colour, and 10 indicated that plants were large with many branches, extensive runner development and dark green in colour. Percent plant cover was estimated using three random placements per plot of a 0.25-m^2 grid. Biweekly ratings and plant cover estimates were taken 6–20 weeks after planting, at which time frost defoliated the plants.

At 6 weeks after planting, 5 plants per plot were selected at random and the entire root system excavated. Roots were cleared and stained, and colonisation by VAM fungi and total root length were estimated by the gridline-intersect method (Giovannetti and Mosse 1980). At 20 weeks after

planting, colonisation was determined on roots from 5 cores (12-cm diam. by 20-cm deep) per plot containing plants and attached roots.

Pot study. Four mycorrhizal treatments [*G. etunicatum* isolates S-312 and S-329, native VAM fungi, and pasteurised (8 h at 88 °C) soil] and three levels of applied P (25, 50 and 75 kg/ha) were evaluated in a greenhouse. The experiment had a randomised complete-block design with 4 replicates per treatment. Limed (4.5 t/ha of $CaCO_3$) Pomona fine sand was allowed to equilibrate for 8 weeks, with the resulting pH at planting ranging from 5.5 in the pasteurised treatment to 5.8 in the S-312 and S-329 treatments. Treatment levels of P, along with K (166 kg/ha) and TEM 300 (22 kg/ha), were mixed thoroughly with the soil prior to planting and 3.1 kg (dry weight) of soil was placed into 15-cm-diam. plastic pots. Soil-P levels (Mehlich I-extractable) ranged from 18–32 mg/kg with additions of 25–75 kg/ha of P, respectively. Inocula were stored at 100 g/kg moisture content at 4 °C for 3 months prior to use. Inocula were applied to non-pasteurised soil by removing 4 cm of soil from each pot, spreading the inocula in a layer, and replacing the upper soil layer. Results of an MPN assay were used to adjust inocula quantities in an attempt to give equal propagule numbers to each treatment. Inocula quantities used were 2, 20 and 20 g of S-312, S-329, and the native inoculum respectively. However, the MPN assay conducted at the time of planting indicated total propagules applied per pot to be 14, 880, and 100 propagules for S-312, S-329, and the native inoculum, respectively.

Twenty scarified and rhizobia-inoculated seeds were placed in each pot. After emergence, plants were thinned to 5 plants per pot. Pots were watered as needed to maintain soil moisture near field capacity. Pots were randomised within blocks and re-randomised biweekly. The average maximum and minimum temperatures during the experiment were 29 and 22 °C, respectively, and mean daily maximum PPFD in the greenhouse was 1159 μmol/m^2/s.

At 28 d after planting, roots were sampled by extracting two soil cores per pot with a 1.5-cm diam. by 10-cm long cork borer. Roots were cleared, stained, and assessed for VAM colonisation as described above. Plants were harvested at 58 d by cutting the shoots at the soil surface. Roots were washed free of soil over a 1.2-mm-mesh screen, blotted dry, and fresh weights were determined. A 0.2-g subsample from each replicate was cleared and stained, and VAM colonisation assessed. Shoots were dried at 70 °C for 48 h, weighed, and ground in a Wiley mill to pass through a 0.84-mm-mesh screen. Tissue samples were ashed at 500 °C for 6 h and then extracted with 0.3 M HCl. Solutions were analyzed for P using the colorimetric method (Murphy and Riley 1962) as modified by Watanabe and Olsen (1965).

Plant combination study

This greenhouse experiment was conducted in pasteurised (8 h at 88 °C) Pomona fine sand to test the effect of VAM inoculation and plant combination on root colonisation. The VAM treatments were *G. etunicatum* S-312 and S-329, native inoculum, and the control. Plant combinations were individual plantings of either *V. parkeri* or *P. notatum*, and a mixed planting of *V. parkeri* and *P. notatum*. Potassium, Fe, Zn, Mn, B, Mo, and Cu were applied in solutions to the limed (pH 5.8), pasteurised soil to supply rates of 60, 2, 1, 1, 0.5, 0.1, and 0.2 mg/kg, respectively. Soil (600 g) was placed into Deepot inserts (656 ml capacity, J.M. McConkey & Co., Inc., Summer, WA, USA) and inocula were placed 4 cm below the soil surface. Prior to placement, inocula amounts were adjusted in an attempt to give approximately equal propagule numbers for S-312, S-329, and the native inoculum by using the results of a MPN assay. Amount of inoculum used was 19, 5, and 50 g, respectively, for S-312, S-329 and the native inoculum. However, the MPN assay conducted at the time of planting indicated total propagules applied per pot to be 912, 490, and 100 for S-312, S-329, and the native inoculum, respectively.

Ten scarified and rhizobia-inoculated seeds were planted per pot and thinned to 2 plants per pot after emergence. Pots were arranged in a randomised complete-block design with 5 replicates. Pots were re-randomised within blocks biweekly. The average maximum and minimum temperatures in the greenhouse during the course of the experiment were 35 and 24 °C, respectively, and mean daily maximum PPFD was 1670 μmol/m^2/s.

VAM colonisation was assessed at 35 d on roots removed from each pot using a 1.5- by 10-cm cork borer. No attempt was made to differentiate roots of *V. parkeri* and *P. notatum* at this time. Plants were harvested 63 d after planting. Roots were

washed free of soil, blotted dry, weighed, and a 0.2-g subsample from each plant was cleared and stained and used to estimate VAM colonisation. Roots of *V. parkeri* and *P. notatum* grown in combination were separated carefully and only attached roots were used to assess colonisation. Shoot tissue was dried, weighed, and analyzed for P as described above.

VAM isolate and soil study

A pot study was conducted using a randomized complete-block design with 3 factors and 4 replicates per treatment. Factors were VAM isolates (*G. etunicatum* isolates S-312 and S-329, and *Gigaspora margarita* isolate S-300), plant species (*V. parkeri* and *P. notatum*), and pasteurized (2450 MHz at 750 W for 4 min; Ferris 1984) soil (Newnan fine sand, and limed and unlimed Pomona fine sand). The Newnan fine sand (sandy, siliceous, hyperthermic Ultic Haplohumod) had been amended previously with composted organic matter and limed to a pH of 7.4, and had a Mehlich I-extractable P of 32 mg/kg and an organic matter content of 38 g/kg. The Pomona fine sand was either unlimed (pH 4.4) or limed at a rate equivalent to 4.5 t/ha of $CaCO_3$ to a pH of 5.8.

Pasteurized soils were placed in supercell containers (164 ml capacity, Ray Leach Conetainer Nursery, Canby, OR, USA), 10 g of inoculum was applied in a layer and covered with an additional 4 cm of soil. Seeds of either *V. parkeri* or *P. notatum* were planted in each pot and thinned to one plant per pot after emergence. Plants were grown in a growth chamber (16 h photoperiod, mean maximum PPFD of 1000 $\mu mol/m^2/s$, maximum and minimum temperatures of 29 and 23 °C, and maximum and minimum relative humidity of 75 and 60%) for 49 d. At harvest, shoot dry weight, root fresh weight, total and VAM colonized root length, and shoot-P concentration were measured. Mycorrhizal colonization was determined on a 0.2-g subsample taken from each replicate. Shoot-P concentrations were determined as described previously.

Statistical analyses

Stand ratings and percent cover data from the field study were analyzed using the repeated measures analysis of the General Linear Model (GLM) procedure (SAS Institute Inc. 1985). Percent cover data were transformed using the arcsine of the square root transformation prior to analysis. Effects of VAM inoculation, soil type, and plant in the plant combination and soil type-VAM isolate studies were analyzed using ANOVA (SAS Institute Inc. 1985). In both these studies, data for *V. parkeri* and *P. notatum* were analyzed in a complete model and separately by plant type. All other data were analyzed using the GLM procedure of SAS.

Results and discussion

Most probable number assays

There was wide variability in propagule numbers both among and within inocula as determined by the MPN assays (Table 1). Isolate S-312 propagule numbers dropped during the initial storage period, but increased in the final two MPN assays. Isolate S-329 had fewer propagules at the time of preparation, but propagule numbers increased with storage. The native inoculum had low propagule numbers in all four assays.

Although some researchers have stressed the importance of equalizing propagule numbers in VAM experiments (Daniels and Skipper 1982; Haas and Krikun 1985), the results of the four MPN assays demonstrate a fundamental problem with this approach. The time required to conduct an MPN assay (6–8 weeks) is sufficient for changes in propagule density to occur (Daft *et al.* 1987). Nonetheless, results of MPN assays should be useful in the interpretation of VAM inoculation studies (Sylvia and Burks 1988).

VAM inoculation in amended Pomona soil

In the field study, plots inoculated with S-312 had higher percent cover by *V. parkeri* ($P = 0.07$) than non-inoculated plots (Figure 1). There were no differences in percent plant cover between *V. parkeri* inoculated with S-329 and the control treatment. Inoculation with VAM fungi did not affect percent VAM colonizationd or stand rating. Phosphorus had no effect on VAM colonization, percent plant cover or stand rating, and there was no interaction between VAM inoculation and P application. Mean VAM colonization was 4.5 and 2.5%, respectively, at 6 and 22 weeks after planting. Stand ratings averaged 4.7 throughout the course of the study.

Table 1. Most probable number of propagules in the VAM inocula (*G. etunicatum* isolates S-312 and S-329, and native fungi) at each MPN harvest. Inocula were prepared on 19 June 1989 and stored at 4 °C until subsequent assays were conducted.

	MPN harvest date			
Inocula	7 Aug 1989	25 Nov 1989	30 May 1990	31 July 1990
S-312	35 (9-133)[1]	7 (2-21)	26 (8-86)	48 (15-158)
S-329	4 (1-11)	44 (13-142)	99 (30-326)	108 (33-358)
Native	4 (1-9)	5 (2-13)	5 (2-13)	2 (1-4)

[1] Most probable number of propagules per g of inoculum with 95% confidence intervals in parentheses (Cochran 1950).

In the pot study, shoot dry weight and VAM colonization were not affected by VAM inoculation or P treatment — mean shoot dry weight for all treatments was 1.3 g/pot and mean percent VAM colonization was very low at both 28 d (0.6%) and 56 d (0.2%). Shoot-P concentrations were affected by VAM inoculation, but not by P application. Shoot-P concentration was greater ($P \leq 0.05$) in the pasteurized control treatment (10 g/kg) than in the three mycorrhizal treatments (6 g/kg). There was no interaction between VAM inoculation and P application for any response variable.

The failure of VAM fungi to colonize *V. parkeri* in both field and pot studies was unexpected. The MPN assay conducted at the time of planting for both studies indicated that the inocula had high propagule densities and sufficient amounts were used to provide adequate propagule numbers for colonization. There were no obvious soil factors that would have suppressed colonization. It is possible that, as an exotic, *V. parkeri* was not able to function as a host for the VAM fungi found in Florida; although such host-fungal specificity in VAM associations is probably rare (Mosse 1975;

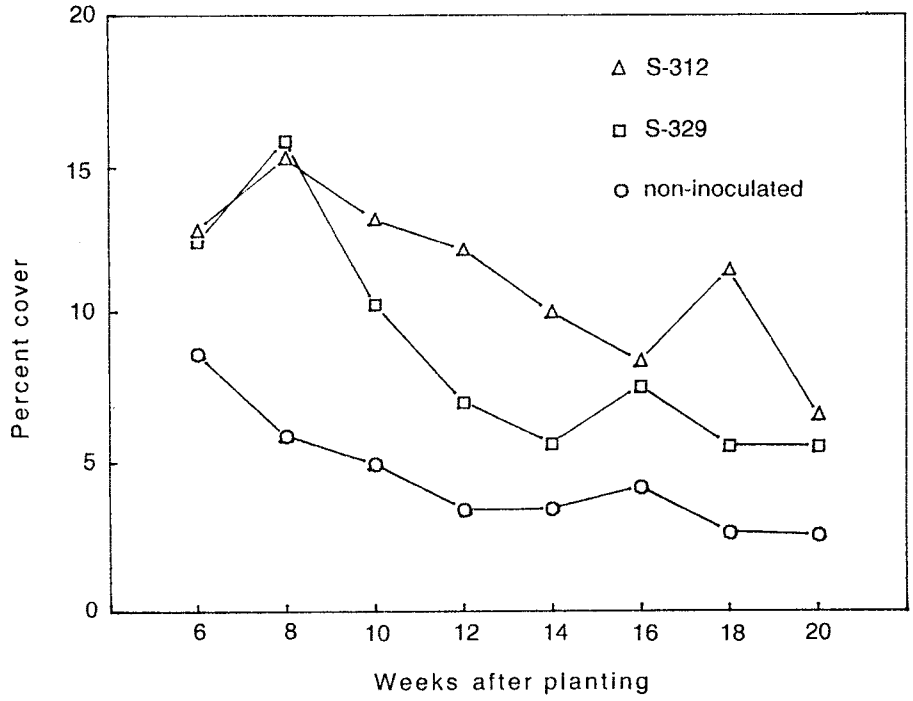

Figure 1. Percent plant cover by *V. parkeri* in response to inoculation with *G. etunicatum* isolates S-312 and S-329. Differences in plant cover between the S-312 and non-inoculated treatments were significant at the P = 0.07 level.

Gianinazzi-Pearson 1984). The most reasonable explanation for poor colonization was that high tissue-P concentrations suppressed colonization. Menge *et al.* (1978), Jasper *et al.* (1979), and Rajapakse *et al.* (1989) demonstrated that tissue-P concentration is an important factor regulating colonization. In general, tissue-P levels above the critical-P level for maximum biomass production of a plant depress VAM colonization. In the pot study, shoot-P levels were well above the levels found by Andrew and Robins (1969a) and Yepez and Blue (1977) to be critical for maximum biomass production by other tropical forage legumes. The high shoot-P content of the plants may be due to the presence of large organic and inorganic P fractions in the soil that were not extracted by the Mehlich-I procedure (O'Donnell *et al.* 1991).

The absence of a mycorrhizal plant-growth response would be expected with the extremely low levels of colonization found in these studies. In contrast, Medina *et al.* (1988a; 1988b; 1990) found good colonization and increased shoot growth of *M. atropurpureum* and *A. americana* inoculated with isolate S-312 using similar rates of P on a P-deficient Florida spodosol (Mehlich I-extractable P of 1 mg/kg). However, these two legumes also responded to added P on the same soil without VAM inoculation.

Although VAM colonization in the field study was low and not different among inoculation treatments, differences were found in plant cover. This suggests that inoculating *V. parkeri* with selected strains of VAM fungi may be important in its establishment and that the indigenous fungi were not effective in the field under the amended soil conditions.

Plant combination study

At 35 d, greatest colonization was found in *P. notatum* inoculated with S-329 (7%) and the *V. parkeri-P. notatum* combination inoculated with S-312 (6%). Colonization of all other planting combinations and inoculations was less than 3%. At 63 d, colonization was greatest in *V. parkeri* grown alone and inoculated with S-329 (Table 2). Within plant combinations there were no differences in shoot dry weight among inocula, but there were differences in shoot-P concentration. With *V. parkeri* grown alone, both the native inoculum and S-312 had greater shoot-P concentration than the control treatment. There were no differences in shoot-P concentrations for *V. parkeri* grown with *P. notatum*. Shoot-P levels were greater for *P. notatum* inoculated with S-329 or the native inoculum than for *P. notatum* grown without added VAM isolates.

Table 2. Effect of inocula (*G. etunicatum* isolates S-312 and S-329, native fungi, and pasteurized control) on VAM colonization, shoot dry mass, and shoot-P concentration of *V. parkeri* grown alone or in combination with *P. notatum*, and *P. notatum* grown alone at 63 d after planting

Inocula	Colonization (%)	Shoot dry weight (g/pot)	Shoot-P conc. (g/kg)
	V. parkeri grown alone		
S-312	1b[1]	0.96a	2.97a
S-329	7a	0.90a	2.71ab
Native	3ab	1.07a	3.03a
Control	0b	0.83a	2.61b
	V. parkeri grown in combination with *P. notatum*		
S-312	3c	0.29b	2.71c
S-329	<1c	0.19b	2.94c
Native	2c	0.26b	3.27c
Control	0c	0.29b	2.69c
	P. notatum grown alone		
S-312	1de	1.54c	1.73ef
S-329	2de	1.50c	1.81de
Native	2d	1.53c	2.01d
Control	0e	1.68c	1.53f

[1] Means within the same column and plant combination followed by the same letter are not different by the LSD mean separation at the 0.05 level.

The poor colonization of *V. parkeri* was similar to results found in the previous VAM inoculation studies. Better VAM colonization was expected in *P. notatum* since this grass is considered a good host plant for VAM fungi (Struble and Skipper 1988). As in the VAM inoculation studies in amended Pomona soil, the inocula used had high propagule densities which were applied in quantities sufficient to colonize host plants. The relatively high tissue-P levels, however, may again have been responsible for the poor colonization of both plant species.

VAM isolate and soil study

Paspalum notatum was included in this study as an indicator plant for VAM colonization, in the event that *V. parkeri* did not become colonized. However, as *V. parkeri* did become colonized, detailed data for *P. notatum* are not presented. Mean colonization of *P. notatum* roots by S-300 over all soils was 37%, while mean colonization by the two *Glomus* isolates was 8%.

For percentage colonization of *V. parkeri*, the main effects of isolate and soil were significant and there was no isolate x soil interaction. Over all soils, isolate S-300 colonized a higher percentage of *V. parkeri* roots (40%) than did S-312 (20%) or S-329 (13%). Over all isolates, there was less percentage colonization in the limed Pomona soil than in the unlimed Pomona or the Newnan soil (Table 3). Total root length and shoot dry weight were also affected by soil, with greatest shoot dry weight and total root length on the limed Pomona and Newnan soils. There was a VAM isolate x soil interaction for colonized root length ($P \leq 0.01$) and shoot-P concentration ($P \leq 0.01$). Colonized root length was greatest on the Newnan soil for S-300 and least in both the limed and unlimed Pomona soil for S-329 (Table 4). Shoot-P concentration for all isolates was greatest in the Newnan soil.

Table 3. Effect of soil type on the percent VAM colonization, total root length, and shoot dry weight of *Vigna parkeri*

Soil	VAM colonization (%)	Total root length (cm/pot)	Shoot dry weight (g/pot)
Newnan	27a[1]	5536a	0.48a
Pomona limed	17b	4803a	0.46a
Pomona unlimed	28a	2397b	0.30b

[1] Means within the same column followed by the same letter are not different by the LSD mean separation at the 0.05 level.

Table 4. Effect of soil type and VAM isolate on colonized root length and shoot-P concentrations of *Vigna parkeri*

Soil	Colonized root length (cm/pot)	Shoot-P concentration (g/kg)
	Isolate S-300	
Newnan	2652a[1]	5.38a
Pomona limed	1383b	1.43c
Pomona unlimed	906cd	2.45b
	Isolate S-312	
Newnan	918cd	5.05a
Pomona limed	704cde	2.03bc
Pomona unlimed	456de	1.82c
	Isolate S-329	
Newnan	1031c	5.08a
Pomona limed	297e	2.54b
Pomona unlimed	296e	2.49b

[1] Means within the same column with the same letter are not different by the LSD mean separation at the 0.05 level.

Colonization levels similar to those observed in other legumes (Medina *et al.* 1988a) were found for *V. parkeri* in this study. Isolate S-300 may be a more effective fungus for inoculation of *V. parkeri*, as indicated by colonization level, than the other isolates in these two soil types that varied in pH and fertility conditions. Sylvia and Schenck (1983) found that this isolate could colonize roots in high-P (199 mg/kg Mehlich I-extractable P) soil and responded to applications of superphosphate (8.7% P) with increased sporulation. Nonetheless, Medina *et al.* (1988b) found it was not an effective isolate for colonization of *M. atropurpureum*.

In this study, colonization was obtained even at shoot-P concentrations >5 g/kg, indicating that high shoot-P concentration was not the only factor that inhibited VAM colonization in the Pomona soil. Another soil factor which has been shown previously to affect spore germination and colonization is pH (Green *et al.* 1987; Graw 1979; Hepper 1984; Abbott and Robson 1985). It is possible that the fungal isolates tested were sensitive to the acidic conditions of even the limed Pomona soil. Dudeck *et al.* (1984) found very low colonization of both *P. notatum* and *Eremuchloa ophiuroides* by *G. etunicatum* at pH 5.8, while colonization of both plants by *G. etunicatum* was greater at pH 7.3. Soil pH may have affected colonization of *V. parkeri* in two ways: by decreasing the infectiveness of the VAM fungi and by increasing the level of available P in the soil. Since a pH between 5.4 and 5.9 is optimum for growth of *V. parkeri* on Florida spodosols (O'Donnell *et al.* 1991), future studies with this plant should include VAM fungal isolates that are known to be infective in this pH range.

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