Importance of indigenous arbuscular mycorrhiza for growth and phosphorus uptake in tropical forage grasses growing on an acid, infertile soil from the Brazilian savannas

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Abstract

A pot experiment was conducted to examine the significance of indigenous arbuscular mycorrhizal fungi (AMF) for growth and phosphorus uptake in some important introduced forage grasses at different soil pH levels in a Brazilian Oxisol. Plants of *Brachiaria brizantha* (BB), *B. decumbens* (BD), *B. humidicola* (HU) and *Panicum maximum* (PM) were grown in a glasshouse for 70 days with or without inoculation with indigenous AMF at 3 levels of initial soil pH (4.3, 5.1 and 6.4). After the growth period, dry weight, concentration of phosphorus (P) and P uptake were determined in both shoots and roots. Inoculation with AMF increased both shoot and root dry weights in BD, BB and PM, but not in HU. Shoot and root P concentrations and total P uptake per pot in all species were improved by mycorrhizal inoculation. Plant growth and P uptake were directly related to soil pH in all grass species. All grass species showed the highest mycorrhizal dependency for dry matter production and P uptake at the lowest soil pH level.

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Introduction

The Brazilian savannas, locally known as "Cerrados", which cover about 2 M km^2 in central Brazil, are one of the most important areas for livestock production in the world (Adamoli *et al*. 1986). Since the 1970s, an estimated 0.5 M km^2 has been sown with tropical grasses of African provenance such as *Brachiaria* species or *Panicum maximum* (Macedo 1995). However, since the soils of the Brazilian savannas are highly acid, low-P soils (Kornelius *et al*. 1979), the productivity and sustainability of the improved pastures are largely affected by the ability of the introduced grasses to extract P from the soil (Rao 2001).

It is generally recognised that the association of arbuscular mycorrhizal fungi (AMF) enhances P uptake by forage grasses in the Brazilian savannas (Mosse 1972; Siqueira *et al*. 1990; Souza *et al*. 1999). However, information on the relationship between the grass-AMF association and soil pH is limited in these savanna regions (Siqueira *et al*. 1990).

Soil pH affects both plant growth and mycorrhizal association. Mosse (1972) indicated that an application of lime to 2 kinds of Brazilian Oxisol, increasing soil pH from 4.7 to 5.0 and from 4.8 to 5.8, improved mycorrhizal effectiveness in *Paspalum notatum* planted in these soils. Moreover, Rheinheimer and Kaminski (1994) reported that the effect of AMF inoculation on the growth and P accumulation of *Paspalum notatum* on a Brazilian Ultisol was maximised, when the soil pH ranged from 5.0 to 5.5. In contrast, Siqueira *et al*. (1990) reported that AMF improved the growth and P uptake of *Brachiaria decumbens* at low and high pH levels, but not at an intermediate level (5.7). Thus, the importance of AMF seems to vary with both soil pH and grass species.

In the previous reports (Mosse 1972; Siqueira *et al*. 1990; Rheinheimer and Kaminski 1994), soil pH had been adjusted by applying limestone or a mixture of calcium carbonate and magnesium carbonate, so that the effects of soil pH

could not be separated from the effects of the applied calcium. In the current study, the effects of indigenous mycorrhiza on growth and phosphorus uptake of 4 tropical grasses were examined at 3 soil pH levels. The soil pH levels were achieved by combining calcium carbonate and calcium chloride, so that the same amounts of calcium were applied in all soil pH treatments. The objectives of the study were to examine: (1) the importance of indigenous AMF for plant growth and P uptake of 4 tropical grass species in the Brazilian savannas; and (2) the effect of soil pH on the grass-AMF association in an Oxisol from the Brazilian savannas.

Materials and methods

Soil and fertilisation

Samples of an Oxisol (dark red Latosol: 38–40% clay) were collected from the top 20 cm of soil in a native savanna area of the National Beef Cattle Research Center of the Brazilian Agricultural Research Corporation in Campo Grande, Brazil (20°27′S, 54°37′W). Soil properties before fertilisation were: 29 g/kg organic matter; 2.25 mg/kg P (Mehlich-I); pH 5.3 in water; 6.8 Ca, 4.6 Mg, 1.1 K and 1.1 Al $mmol_c$/kg.

The soil, containing 21% water, was fumigated for 48 h in a concrete chamber with 0.1mL/kg Bromex (mixture of methylbromide and chloropicrin) to eliminate mycorrhizal fungi. After the fumigation, the soil was divided into 3 parts, one for each soil pH treatment. For the low pH treatment, 2.94 g/kg $CaCl_2{\cdot}2H_2O$ was applied to the soil; in the medium pH treatment, 1.5 g/kg $CaCO_3$ and 0.73 g/kg $CaCl_2{\cdot}2H_2O$ were applied; and, in the high pH treatment, 2.0 g/kg $CaCO_3$ was applied. Overall, 0.8 g/kg Ca was applied as $CaCO_3$ or $CaCl_2$ to all soil pH treatments. At the same time, all treatments received 12.5 mg/kg P as superphosphate. After 2 weeks of incubation, samples of soil (1.8 kg dry weight) were placed in individual pots. Soil pH at this time was 4.3, 5.1 and 6.4 for the low, medium and high soil pH treatments, respectively.

Inoculation with arbuscular mycorrhizal fungi

Soil samples (approximately 10 kg) were collected each from *Brachiaria brizantha*, *B. decumbens*, *B. humidicola* and *Panicum maximum* pastures. Coarse debris and rocks were removed from the samples with a 2 mm sieve, and then 5 kg of each sample was weighed for collecting the indigenous arbuscular mycorrhizal inoculum assemblages using a wet-sieving and decanting technique (Brundrett *et al*. 1996). Each aliquot of approximately 200 g of soil was mixed in 2 L of water and decanted through a 105 µm sieve. This procedure was repeated several times until the water was clear for each 200 g soil sample. All sievings from the 4 pasture soils were mixed together to obtain an inoculum suspension of 1 L. Based on microscopic observation, the inoculum contained *Gigaspora* spp., *Scutellospora* spp., *Glomus* spp., *Acaulospora* spp. and *Entrophospora* spp.

At the same time, only the first washing of the first 200 g sample was collected each from the 4 pasture soils. The 4 washings were mixed and filtered through a filter paper (Whatman No 5) for applying microbial populations other than AMF to the non-mycorrhizal treatment (Mosse 1972; Manjunath *et al*. 1989).

In the mycorrhizal treatment, 4 small holes were made in the soil surface of each pot, and 2.5 mL of inoculum was placed in each hole: each pot received the sievings from 200 g of soil. In the non-mycorrhizal treatment, 1.25 mL of the filtered leachate was added to each hole.

Experiment

Seeds of *Brachiaria brizantha* cv. Marandu (BB), *B. decumbens* cv. Basilisk (BD), *B. humidicola* cv. Humidicola (HU) and *Panicum maximum* cv. Tanzania (PM) were surface-sterilised in concentrated H_2SO_4 (97%) for 1 min, and washed in several rinses of distilled water. The seeds of each species were sown into sterilised soil on different plastic plates. The plastic plates were watered daily with distilled water, and 10 days after sowing, germinated plants were transplanted into the experimental pots in which mycorrhizal or non-mycorrhizal treatments were applied.

After transplanting, each pot received a further application of a solution containing: 26.5 mg/kg K_2SO_4, 68.7 mg/kg $MgSO_4{\cdot}7H_2O$, 7.3 mg/kg $ZnSO_4{\cdot}7H_2O$, 1.3 mg/kg $CuSO_4{\cdot}5H_2O$, 1.4 mg/kg $Na_2B_4O_7{\cdot}10H_2O$, 0.3 mg/kg $Na_2MoO_4{\cdot}2H_2O$ and 54.4 mg/kg urea (24.5 mg/kg N), as a basic fertiliser. The equivalent of 14.8 mg/kg S was applied in the above fertiliser.

The experiment was carried out in a glasshouse for 70 days, employing a factorial design (4 grasses × 3 pH levels × 2 AMF treatments) with 8 replicates. Distilled water was added

daily to maintain soil moisture at approximately 0.6 times total pore volume, which corresponded with 29% of water to dry soil. There was no leaching from the pots. To prevent dispersal of soil particles and cross-contamination of AMF, each pot was watered carefully on an electronic balance. Moreover, since this experiment was conducted during the dry season (winter), windows were closed to prevent wind from entering the glasshouse and dispersing soil particles. Mean temperature and mean relative humidity in the glasshouse during the growth period were 26.6°C and 52%, respectively.

At the end of the 70-day growth period, above-ground parts were harvested, weighed fresh and then oven-dried at 60°C for 2 days for dry weight determination. Remaining parts (base of shoot and roots) from 5 replicates were washed carefully, separated and oven-dried to determine dry weight of each component. In this report, a 'shoot' consists of above-ground parts including the shoot base. For the remaining 3 replicates, under-ground parts were separated carefully from the soil, using approximately 3 L of water, and the soil collected and dried in the glasshouse for chemical analysis. Fresh weights of the shoot bases and roots were measured, and then for each plant, the mass of roots was separated vertically into 3 sub-samples for determination of AMF infection, root length and dry matter, ensuring that the total lengths of roots were included in each sample. The rate of AMF colonisation was measured according to Giovannetti and Mosse (1980). Subsamples (0.01–1.22 g fresh weight) were cleared with KOH (100 g/L) for 2 hours in a waterbath at 90°C, acidified in HCl (2%) for 0.1 hour and stained with trypan blue in lacto-glycerol for 0.1 hour (Brundrett *et al*. 1996). AMF colonisation was estimated using the grid-line intersection method at 100 intersections (Giovannetti and Mosse 1980). Root length was measured according to Crestana *et al*. (1994), using scanned digital images.

Soil pH was measured on the pot soils dried in the glasshouse and the following chemical analyses were performed: exchangeable cations (K, Ca and Mg), available P (Mehlich-I) and 1N-HCl-extractable Al (Salinas and Gracia 1985).

P concentration in the plants was determined using composite samples in each treatment, because the plant fragments were so small in some treatments. In the low pH treatment, a single whole plant sample (roots + shoots) was finely milled by passing through a 2 mm sieve. In the medium and high pH treatments, the samples for each plant component from each 4 replicates were mixed, giving 2 samples for each treatment for both shoots and roots. The P concentrations in shoots and roots were determined using a colorimetric method (Salinas and Gracia 1985). By multiplying mean P concentration in shoots and roots by their dry weight for each pot, the amount of P absorbed by the plants was estimated for each replicate.

Mycorrhizal dependency (Saif 1987) was determined by expressing total dry weight (or total P uptake) of a mycorrhizal plant as a percentage of the total dry weight (or total P uptake) of a non-mycorrhizal plant.

Data were subjected to analysis of variance using SAS ANOVA analysis (SAS Inst. Japan 1990). A 3-factor statistical design was used; the number of replicates was 8 for shoot and root dry weight, shoot:root ratio and P uptake, and 3 for rate of AMF infection, root length and soil chemical properties. For P concentrations in shoots and roots, the results were statistically analysed only for the pH 5.1 and 6.4 treatments with 2 replicates. Means were separated using Tukey's multiple comparison procedure, with all significant differences reported in this paper at $P \leq 0.05$.

Results

Soil analysis

Chemical properties of the pot soils at the end of the experiment are shown in Table 1. Soil pH was 4.2, 4.5 and 5.1 in the low, medium and high soil pH treatments, respectively, at the end of the experiment. Concentrations of available P, K, Mg and Al of the soils decreased with an increase in soil pH, while Ca concentration was not affected by pH level.

Table 1. Results of soil analysis at the end of the experiment.

Soil pH treatment	pH	P	K	Ca	Mg	Al
		(mg/kg)		($mmol_c$/kg)		
Low	4.22 c[1]	2.49 a	1.33 a	39.0 a	8.15 a	6.82 a
Medium	4.53 b	2.35 a	0.87 b	42.9 a	6.76 b	2.95 b
High	5.10 a	2.06 b	0.53 c	42.4 a	5.13 c	1.66 c

[1] Values within the same column followed by the same letters are not significantly different at the 5% level.

Plant growth

Both mycorrhizal and soil pH treatments significantly ($P < 0.05$) affected shoot, root and total dry weights of the grass species (Table 2). While inoculation with AMF increased plant growth overall, the responses differed among the species, with HU failing to respond to inoculation. Shoot, root and total dry weights increased as pH increased. However, between the mycorrhizal treatment and soil pH, there was a significant interaction for root dry weight and total dry weight. AMF increased root dry weight significantly only at low pH, and total dry weight only in the low and high pH treatments.

Table 2. Effects of mycorrhizal inoculation and soil pH on shoot dry weight, root dry weight, total dry weight and shoot: root ratio in the 4 grass species examined.

	Shoot dry weight		Root dry weight	
	–AM[1]	+AM[1]	–AM	+AM
	(g/pot)		(g/pot)	
Species				
BB[2]	1.41 Bb[3]	2.54 Aab	0.49 Bb	0.64 Ab
BD	1.23 Bb	2.52 Aab	0.41 Bb	0.67 Ab
HU	1.88 Aa	2.36 Ab	0.99 Aa	0.91 Aab
PM	1.96 Ba	3.08 Aa	0.85 Ba	1.21 Aa
Soil pH				
Low	0.11 Bc	0.61 Ac	0.04 Bc	0.13 Ac
Medium	1.81 Bb	2.48 Ab	0.63 Ab	0.65 Ab
High	2.94 Ba	4.79 Aa	1.39 Aa	1.80 Aa
Mean	1.62	2.63	0.69	0.86

	Total dry weight		Shoot : root ratio[4]	
	–AM	+AM	–AM	+AM
	(g/pot)			
Species				
BB	1.89 Bb	3.18 Ab	3.52 Bab	4.58 Aa
BD	1.65 Bb	3.19 Ab	4.11 Aa	4.22 Aa
HU	2.87 Aa	3.27 Ab	2.01 Bc	4.37 Aa
PM	2.82 Ba	4.29 Aa	2.88 Bbc	3.63 Aa
Soil pH				
Low	0.15 Bc	0.74 Ac	3.59 Ba	4.83 Aa
Medium	2.44 Ab	3.13 Ab	3.41 Ba	4.51 Aa
High	4.33 Ba	6.58 Aa	2.39 Ba	3.26 Ab
Mean	2.31	3.48	3.13	4.20

[1] Treatments: –AM = no mycorrhiza applied, +AM = mycorrhiza applied.
[2] Abbreviations for grass species: BB, *B. brizantha*; BD, *B. decumbens*; HU, *B. humidicola*; PM, *P. maximum*.
[3] Within parameters, values within the same column (lower case) or row (upper case) followed by the same letter are not significantly different at the 5% level.
[4] Values are means of the individual shoot : root ratios for all pots within species and soil pH treatments.

Total dry weight of each grass species at the 3 pH levels is shown in Figure 1. In HU, the mycorrhizal effect varied with soil pH: there was no effect in the low pH treatment, a negative effect in the medium pH treatment, and a positive effect in the high pH treatment. In BD, inoculation with AMF had no effect on total dry weight for the medium pH treatment.

SR ratio (shoot dry weight:root dry weight) was increased significantly by mycorrhizal inoculation at all pH levels (Table 2). The mean increase in SR ratio due to inoculation was: BB 30%; BD 3%; HU 117%; and PM 26%, with the response in HU greater than that in the other species.

Average percentage of the roots infected by AMF varied between 33% and 52% in the inoculated treatment (Table 3). In the un-inoculated plants, 5.6–15.6% of roots were infected by AMF.

Table 3. Percentage of roots infected by AMF and root length per pot for each grass species and soil pH treatment.

	Percentage of roots infected by AM		Root length	
	–AM[1]	+AM[1]	–AM	+AM
	(%)		(m/pot)	
Species				
BB[2]	15.6 Ba[3]	50.8 Aa	37.1 Abc	28.7 Ab
BD	11.5 Bab	44.4 Aab	30.3 Ac	35.7 Ab
HU	10.4 Bab	52.2 Aa	93.2 Aa	43.4 Bb
PM	5.6 Bb	33.2 Ab	61.3 Bb	88.7 Aa
Soil pH				
Low	10.1 Ba	31.4 Aa	5.9 Bb	23.6 Ab
Medium	14.6 Ba	49.7 Aa	47.5 Ab	31.9 Ab
High	7.6 Ba	54.3 Aa	113.0 Aa	91.9 Aa
Mean	10.8	45.1	55.5	49.1

[1] Treatments: –AM = no mycorrhiza applied, +AM = mycorrhiza applied.
[2] Abbreviations for grass species: BB, *B. brizantha*; BD, *B. decumbens*; HU, *B. humidicola*; PM, *P. maximum*.
[3] Within parameters, values within the same column (lower case) or row (upper case) followed by the same letter are not significantly different at the 5% level.

Effect of inoculation with AMF on average root length per pot was different among the 4 grass species (Table 3). At the extremes, mycorrhizal treatment increased root length per pot by 45% in PM, while it reduced root length by 53% in HU. There was no significant effect on root length of BB and BD. Root length increased as pH increased, with a strong tendency for a positive correlation between root length and soil pH in all grass species.

P concentration and uptake

Mycorrhizal inoculation increased P concentrations in shoots and roots of all grass species and at all pH levels (Table 4) plus total P uptake by

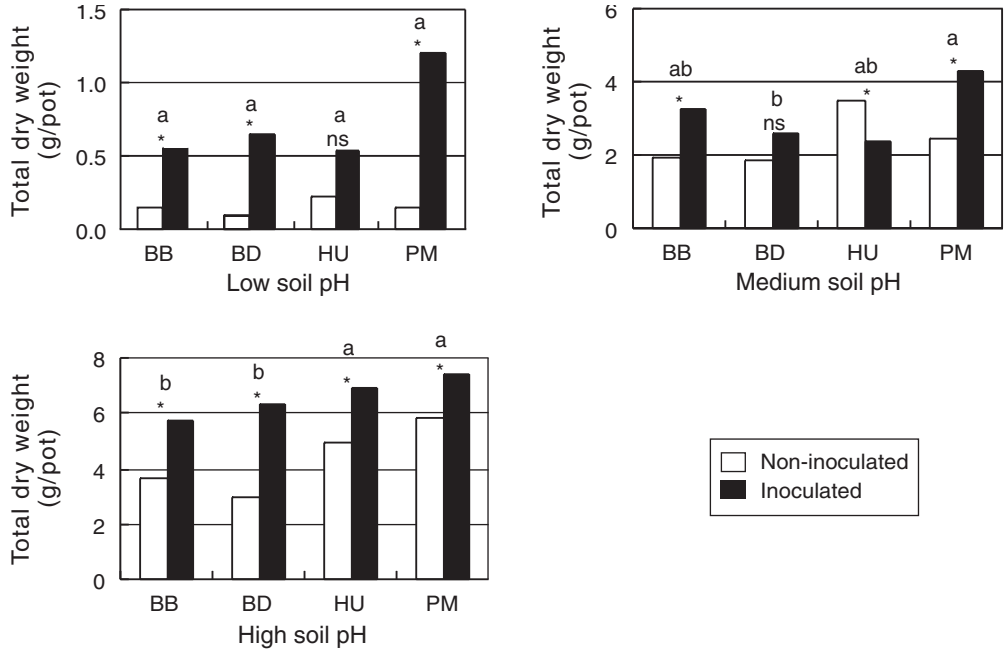

Figure 1. Total dry weight of 4 grass species affected by mycorrhizal inoculation and soil pH.
Abbreviations for grass species: BB, *B. brizantha*; BD, *B. decumbens*; HU, *B. humidicola*; PM, *P. maximum*.

Note: Asterisks on the columns indicate significant differences between the inoculation treatments. Within pH levels, differences between grass species are indicated by different letters (a, b), according to Tukey's Range Test at the 5% level.

Table 4. Effects of mycorrhizal inoculation and soil pH on P concentration in shoot and root, and total P uptake.

	P concentration in shoot		P concentration in root		Total P uptake	
	–AM[1]	+AM[1]	–AM	+AM	–AM	+AM
	(g/kg)		(g/kg)		(mg/pot)	
Species						
BB[2]	0.37 Bc[3]	1.27 Aa	0.44 Bab	0.88 Aa	0.86 Bb	3.60 Aa
BD	0.48 Ba	1.05 Aab	0.48 Ba	0.86 Aa	0.91 Bb	2.91 Aa
HU	0.47 Bab	1.20 Aa	0.44 Bab	0.92 Aa	1.53 Ba	3.24 Aa
PM	0.41 Bbc	0.89 Ab	0.38 Bb	0.73 Aa	1.29 Ba	3.34 Aa
Soil pH						
Low	0.43[4]	0.96[4]	—	—	0.07 Bc	0.70 Ac
Medium	0.42 Ba	1.16 Aa	0.45 Ba	0.93 Aa	1.06 Bb	3.42 Ab
High	0.44 Ba	1.04 Aa	0.42 Ba	0.77 Ab	2.32 Ba	5.70 Aa
Mean	0.43	1.10	0.44	0.85	1.15	3.27

[1] Treatments: –AM = no mycorrhiza applied, +AM = mycorrhiza applied.
[2] Abbreviations for grass species: BB, *B. brizantha*; BD, *B. decumbens*; HU, *B. humidicola*; PM, *P. maximum*.
[3] Within parameters, values within the same column (lower case) or row (upper case) followed by the same letter are not significantly different at the 5% level.
[4] Shoot and root samples were mixed for each grass species.

all species and at all pH levels. Total P uptake increased as pH increased in both inoculated and un-inoculated treatments.

A significant AM × grass species interaction was observed in P uptake. In the non-mycorrhizal plants, total P uptake by BB and BD was smaller than that by HU and PM, whereas in the inoculated treatment, level of P uptake was similar for all grass species.

Mycorrhizal dependency

Mycorrhizal dependency was highest at the low pH treatment for all grass species both for total dry weight and P uptake (Table 5). For BB and

PM, increase in soil pH decreased their mycorrhizal dependency, while, for HU, the value was least at the medium soil pH treatment both for total dry weight and P uptake. BD behaved like HU in terms of total dry weight, and like BB and PM for P uptake. Since the mycorrhizal effect on total dry weight was negative for HU at the medium pH treatment, its mycorrhizal dependency was less than 100. Comparing the grass species, PM showed the highest mycorrhizal dependency in the low pH treatment, but the lowest in the high pH treatment. In the low and medium soil pH treatments, HU showed the lowest mycorrhizal dependency among the 4 species.

Table 5. Mycorrhizal dependency for total dry weight and P uptake in the 4 grass species examined.

	Soil pH treatment		
Species	Low	Medium	High
	Mycorrhizal dependency (%)		
for total dry weight			
BB[1]	**403**[2]	**170**	**158**
BD	**679**	139	**213**
HU	236	**68**	**141**
PM	**798**	**173**	**126**
for P uptake			
BB	**1184**	**527**	**350**
BD	**1047**	**359**	**287**
HU	**496**	**173**	**225**
PM	**2044**	**405**	**172**

[1] Abbreviations for grass species: BB, *B. brizantha*; BD, *B. decumbens*; HU, *B. humidicola*; PM, *P. maximum*.
[2] Within parameters, values in bold characters indicate that values in mycorrhizal plants were significantly different from those in plants not inoculated.

Discussion

Mycorrhizal effect on growth and P uptake by grass species

The present study indicated that the indigenous AMF enhanced the growth of these tropical grasses planted on the Brazilian Oxisol, which supports the previous studies by Mosse (1972), Siqueira *et al*. (1990) and Souza *et al*. (1999). These responses occurred despite the fact that roots of the non- inoculated plants were also infected by AMF. Salinas *et al*. (1985) also reported that, though eradication of native mycorrhizal fungi from pot soil was incomplete, inoculation with *Glomus manihotis* showed stimulation in growth of *Andropogon gayanus* and *Pueraria phaseoloides* at 70 days after planting, but not at 100, 130 and 160 days. In the present study, plant growth was measured at 70 days after planting.

A number of possible reasons exist for the presence of AMF in the non-inoculated plants. Cross-contamination in the glasshouse is one possibility. However, dispersal of mycorrhizal fungi through watering or air currents was prevented, so it is highly improbable that the high rate of mycorrhizal infection (5.6–15.6%) was caused by cross-contamination. Another possible reason is incomplete eradication of the native mycorrhizal fungi. Fumigation using methyl bromide is less effective in clay soil than in sandy loam or loamy sand (Mende 1982). We used a heavy clay soil, and the dosage of fumigant may have been insufficient to eliminate 100% of the native mycorrhizal fungi.

The leachate applied to the non-mycorrhizal plants is a further possible source of contamination. According to the method of Mosse (1972) and Manjunath *et al*. (1989), filtered leachate was applied to pots in the non-mycorrhizal treatment to maintain the microbial populations other than AMF. Those authors did not observe contamination caused by the leachate application. However, the spore size of AMF varies from 5 μm to 1000 μm (Brundrett *et al*. 1996), and it is possible that some small spores of AMF passed through the filter paper. Thus, insufficient eradication of the native mycorrhizal fungi and the application of the leachate seem the most likely reasons for mycorrhizal infection in the non-inoculated plants.

In a study of mycorrhizal dependency among 24 forage species including BD, BB, HU and PM using a Colombian Oxisol (pH 4.5), Saif (1987) found that mycorrhizal dependency was in the order: BD > BB > HU > PM. This result is similar to our finding at the highest pH but conflicts with the order in our study in the low and medium soil pH treatments. In the two studies, the same cultivars of BD, BB and HU were used, but Saif (1987) used accession CIAT 604 of PM, while we used Tanzania. This is one possible explanation for why PM responded to the mycorrhizal inoculation differently in the 2 experiments.

Mycorrhirzal inoculation influences root structure of plants, for example, by reducing root length in perennial ryegrass (*Lolium perenne*) (Fitter 1977) and paprika plant (*Capsicum frutescens* var. *Passion*) (Wong and Lin 2000). In our study, mycorrhizal inoculation reduced root length of HU, but increased that of PM. Since mycorrhizal inoculation did not affect root weight of HU, the roots of this species must have been thickened as a result of inoculation.

Effect of soil pH on the performance of AMF and grass species

In this study, differences in soil pH between the different treatments initially (pH 4.1, 5.3 and 6.4) were reduced significantly after 70 days of growth (pH 4.2, 4.5 and 5.1). We have no data on how rapidly these changes occurred. However, since the differences in magnitude of plant parameters at the different pH levels were so marked, we consider that pH differences must have persisted for a significant part of the experimental period and would be a fair reflection of the true effect of soil pH differences. In fact, our results may underestimate the true differences attributable to differences in soil pH.

Growth of the 4 grass species was reduced markedly at lower soil pH. Since Ca concentration of the pot soils was similar for all soil pH treatments, the growth reduction could not be due to Ca deficiency, but must be due to soil acidity itself. In general, soil acidity induces high Al concentration in soil, which inhibits root expansion of plants, resulting in reduced growth (Sanchez and Isbell 1978). Although $CaCl_2$ was used in these treatments to obtain the desired soil pH levels, we consider it unlikely that the presence of chloride would have inhibited root growth in those treatments. There is relatively little information concerning the effect of Cl on growth of the tropical grasses used in this study. Mergulhao *et al*. (2002) examined the influence of NaCl on *Brachiaria humidicola* plants, and reported that the critical application level of NaCl was approximately 1.1 g/kg, corresponding to a Cl level of 0.66 g/kg. In our study, the Cl level in the untreated soil was not analysed, but in a similar Brazilian Oxisol, Pavan *et al.* (1987) reported Cl at only 0.05 g/kg soil. Thus, it is unlikely that, in the medium pH treatment, the Cl addition of 0.35 g/kg soil would raise total soil Cl to the reported 0.66 g/kg critical level affecting growth. Therefore, we consider that the growth reduction on the lower pH treatments observed in this study was caused by soil acidity, and not by the existence of excess Cl or Ca deficiency in the soils.

Soil pH affected the mycorrhizal dependency of all the examined species, with mycorrhizal dependencies for dry matter production and P uptake highest at low pH. It appears that, since root expansion was inhibited by soil acidity, the significance of AMF for growth and P uptake increased in the low pH treatment. This would explain why the mycorrhizal dependency of the grass species was highest at the low pH treatment.

A notable phenomenon was that mycorrhizal dependency for total dry weight was least in the medium pH treatment for BD and HU. For HU, the mycorrhizal treatment reduced growth in the medium pH treatment, but stimulated P uptake. Reviewing studies on the mycorrhizal effect for tropical forage grasses planted in Brazilian Oxisols or Colombian Oxisols, Siqueira *et al*. (1990) observed that AMF had little effect on shoot growth of *B. decumbens* at the intermediate lime rate at which soil pH in water was 5.7. On the other hand, in Japan, Tsuchida and Nonaka (2003) indicated that inoculation with *Glomus clarum* had a growth-promoting effect on two cultivars of orchardgrass (cvv. Aonami and Kitamidori) planted in a mixture of Andosol and sandy soil, but a growth-inhibitory effect on two other cultivars (cvv. Akimidori and Natsumidori). These results indicate that inoculation with AMF will not always prove beneficial, and that mycorrhizal dependency for growth was determined by an interaction between soil pH and grass species or cultivar.

Between AMF and a host plant, there is an exchange of photosynthetically derived carbon compounds and mineral nutrients (Brundrett *et al*. 1996), with the host plant providing the photosynthetic products for both AMF and its own roots to enable absorption of mineral nutrients. Therefore, partitioning of the photosynthetic products to its own roots is more beneficial than to AMF in some cases where the mycorrhizal effect on growth of the host plant is unclear. Since BD and HU are better adapted to the infertile acid soils than BB and PM (Rao *et al*. 1996), it is possible that, in the medium soil pH treatment in our study, root expansion in BD and HU was not limited by soil acidity and could be independent of association with AMF. This may be a reason why mycorrhizal dependency of BD and HU was low at medium soil pH.

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