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Arbuscular mycorrhizal inoculation and superphosphate application influence plant development and yield of coffee in Brazil

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Abstract This paper reports a 6-year field study of the effects of mycorrhizal pre-colonization of coffee seedlings on initial crop development and coffee bean yield in a low-fertility Oxisol amended with superphosphate (P) at planting. The experiment included five P rates (0, 20, 40, 80 and 160 g plant $^{-1}$ P_2O_5) combined with seven fungal treatments [non-mycorrhizal control, pre-colonization with a mix of *Glomus clarum* and *Gigaspora margarita* (CM) and with five isolates of *Glomus etunicatum*]. Inoculated and non-inoculated outplants were raised under glasshouse conditions, transplanted into the field in January 1989 and monitored until July 1995. Plant height and stem diameter were greatly enhanced by P application and were higher in mycorrhizal seedlings than in controls up to 19 months after transplanting (MAT) but were not different at 26 MAT. Inoculation effects on tree canopy diameter were significant up to 26 MAT, at which time mycorrhizal colonization was high (43–55%), but did not differ amongst plants, regardless of whether or not the plants had been pre-colonized at the nursery stage. Root colonization and spore number in the soil were reduced by high P rates at 26 MAT. The first bean yield (1991) was highly enhanced by P and all pre-colonization treatments (38% increment over control) and these factors showed a significant interaction. Three isolates of *G. etunicatum* showed yield enhancements above 50%. The P rate for maximal yield was 207 g plant $^{-1}$ P_2O_5 for non-pre-colonized and approximately 100 g plant $^{-1}$ for pre-colonized plants. For this harvest, the mycorrhizal biofertilizer effect was equal to 254 kg ha $^{-1}$ P_2O_5. In subsequent years, pre-colonization effects were reduced and inconsistent. In 1992, 1993 and 1995, yield was affected by P but not by mycorrhizal inoculation. In 1994 there was a P versus mycorrhiza interaction and CM and *G. etunicatum*-Var gave higher yields than non-precolonized plants. Considering accumulated yield for this 5-year period, P application resulted in high yield increment in all treatments, whereas pre-colonization effects were extremely diminished. However, despite inconsistency amongst mycorrhizal treatments, pre-colonization effects were detected at the fifth harvest in some fungal treatments. Based on the total yield of five harvests, maximal productivity was achieved with CM at 20 g plant $^{-1}$ P_2O_5 and with CM and *G. etunicatum*-Var at the highest P rate. Diminishing mycorrhizal effects over time are related to colonization of non-precolonized seedlings by the indigenous fungi and to the reduced external P requirement of the mature crop. If adequate phosphorus is applied at planting, pre-colonization of outplants with selected arbuscular mycorrhizal fungi enhances early crop development and productivity of coffee in low-fertility soils of Brazil.

Key words Endomycorrhizae · *Coffea arabica* L. · Tropical agriculture · Phosphorus nutrition · Soil fungi · Field inoculation · Mycorrhizal fungi

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Introduction

Coffee (*Coffea* sp.) is a tropical crop of 75 countries with a total production close to 100 million (60 kg) bags. This agribusiness is estimated at US$ 35 billion annually, which is socially and economically important for the constantly growing population of the tropics. Brazil is the largest producer of *Coffea arabica,* with an annual production of 28 billion bags, and is also the major coffee exporter, followed by Colombia, Central American Countries, Indonesia and Africa. In Brazil, coffee agro-ecosystems extend over an area of 2.3 million ha, with over 4 million trees cultivated as a monocrop. Generating approximately US$ 2.5 billion in exports yearly; coffee is responsible directly or indirectly for 10 million jobs (Minas Gerais-Secretaria do Estado da Agricultura 1995).

Although arbuscular mycorrhiza (AM) was discovered in the last century, scientific and commercial interest in this symbiosis only began in the late 1950s, when benefits for plant growth and nutrition were first demonstrated (Gerdemann 1968). Despite a tremendous volume of research, especially in the last 3 decades, there has been no major advance in the large-scale application of AM technology. It is necessary to expand field experimentation and to assess the cost/benefit of crop inoculation with AM fungi. Considering the mechanisms of mycorrhizal benefits to crops and soil and climate characteristics, AM are especially important for tropical agriculture (Siqueira and Saggin-Júnior 1995), in particular for Brazil and other countries with highly weathered, low-fertility soils. To be productive, such soils must be correctly fertilized, mainly with P, at planting (Malavolta and Kliemann 1985).

It has been known since the late 1800s that coffee plants are heavely mycorrhizal (Janse 1897). Recent studies have confirmed this finding, shown the diversity of AM fungi (Siqueira et al. 1995), elucidated growth effect mechanisms (Saggin-Júnior et al. 1994; Siqueira and Colozzi-Filho 1986) and demonstrated the importance of AM symbiosis to coffee in low-fertility soils (Lopes et al. 1983; Sieverding 1991). Mycorrhizal coffee seedlings grow much faster, exhibit improved nutrition and gave higher yields than those without mycorrhizae at the nursery stage (Siqueira et al. 1993). Mycorrhizal growth effects are primarily nutritionally mediated and are inversely related to improved soil fertility, especially available soil P, which affects mycorrhizal dependency and fungus symbiotic effectiveness (Colozzi-Filho et al. 1994; Saggin-Júnior et al. 1994; Saggin-Júnior and Siqueira 1995). Studies of inoculation effects on coffee yield in our laboratory since 1986 indicate that in low-fertility soils, as in the cerrado soils of Brazil, pre-colonization of coffee outplants increases yield by as much as 74%. However, there is only an effect if adequate soil P is available (Siqueira et al. 1993). Although these results were encouraging, only one superphosphate rate was studied. Furthermore, P-mycorrhizal fungus interactions have been well studied in controlled environments (Lopes et al. 1983; Saggin-Júnior et al. 1994; Saggin-Júnior et al. 1995; Saggin-Júnior and Siqueira 1995; Siqueira and Colozzi-Filho 1986) but not in long-term field experiments. In this study, we examined effects on plant development and productivity over 6 years in a low-fertility soil of the pre-colonization of coffee outplants by six AM fungi under five rates of P addition at planting.

Materials and methods

Site characteristics

This study was conducted in a clayey, low-fertility, high-phosphate-adsorbing, Red Yellow-Latosol (Oxisol) on the experimental farm of EPAMIG in Patrocínio at 934 m elevation in the cerrado area of Minas Gerais State. The climate at the experimental site is classified as Cwa (Köppen system), with a mean annual temperature of 21.8 °C and an annual rainfall of 1372 mm. Before planting, the soil was under the original cerrado vegetation and had a pH (water) of 4.8, 1 and 47 mg kg^{-1} of P and K, respectively, by Mehlich I extraction, and 0.8, 0.2 and 0.2 meq/100 cm^3 of Ca, Mg and Al, respectively, by 1 N KCl extraction. Spore extraction from the experimental site, revealed the presence of *Gigaspora margarita* and *Acaulospora scrobiculata.* The site was limed by broadcasting 3 t ha^{-1} of dolomitic limestone, 60 days before planting.

The experiment

The experiment consisted of 35 treatments of a two-way factorial design (5 P application rates × 7 inoculations treatments). We imposed each treatment on a plot of 18 plants in 3 rows of 6 plants each with 3.5 m between rows and 1 m between plants within a row. The outermost 14 of these plants served as a buffer against edge effects. We took data on the central 4 plants and used the totals for these 4 plants (adjusted to kg ha^{-1}) as the results per plot. We replicated each treatment 4 times, once in each of 4 randomized complete blocks. Thus, the entire experiment involved 140 plots containing a total of 2520 plants, of which we used 560 for quantitative results. The site was quite homogeneous in terms of soil properties and the blocking of treatment plots was based on site position in relation to winds and topography. P treatments consisted of the application of triple superphosphate (40% P_2O_5) at 20, 40, 80 and 160 g $plant^{-1}$ P_2O_5. Superphosphate was throughly mixed with the planting soil. P rates were based upon extractable P of the original soil and on the crop recommendation of 80 g $plant^{-1}$ P_2O_5 for this particular soil (Guimarães 1986). In addition to P treatments, all plants received 300 g $plant^{-1}$ of agricultural gypsum. Extractable P (Mehlich I) at planting for the different superphosphate application rates were 6, 36, 180, 225 and 650 mg kg^{-1} P, respectively.

Seedling inoculation and cultivation

Coffee outplants with the different fungal treatments were raised in a glasshouse at the Department of Soil Science, Federal University of Lavras (UFLA), Lavras (MG) according to procedures used elsewhere in Brazil. Seeds of *Coffea arabica* cultivar "Mundo Novo LCP 379/19" were disinfected with 1% sodium hypochlorite for 5 min and placed to germinate in sterile vermiculite. Seedlings with cotyledon leaves were transferred to plastic bags (7 × 17 cm), filled with a fumigated (Bromex-Bromine Compounds Ltd., Beer Sheva, Israel) soil mix composed of 0.85 m^3 of subsoil plus 0.15 m^3 of cow manure. The soil mix was supplemented with 1.6 kg of simple superphosphate (18% P_2O_5) and 0.20 kg of KCl per m^3. Partial chemical analysis of this mix showed a pH (water) of 5.2 and 31 and 156 mg kg^{-1} of Mehlich I extractable P and K, respectively.

For mycorrhizal pre-colonization, seedlings were inoculated with spore suspensions at the time of transfer to plastic bags. Approximately 300 spores per seedling were delivered onto the roots. Clean spore suspensions were obtained by wet sieving and centrifugation (Gerdemann and Nicolson 1963) of soil inoculum from brachiaria grass (*Brachiaria decumbens* Stapf Prain) pot cultures. Microbiota in all treatments were standardized by adding inoculum fungus-free filtrate to control seedlings. Inoculant fungi were a mixture of *Glomus clarum* Nicolson and Schenck and *Gigaspora margarita* Becker and Hall, designated as CM, and 5 isolates of *Glomus etunicatum* Becker and Gerdemann, originally isolated from coffee agro-ecosystems in Southeastern Brazil (Fernandes and Siqueira 1989) and previously selected as effective for coffee (Colozzi-Filho et al. 1994). These fungi were maintained and multiplied in pot cultures in our laboratory. The isolates were designated according to their origin as follows: *Var* Varginha, *TP* Três Pontas, *Lav* Lavras, *Par* São Sebastião do Paraíso, *Pat* Patrocínio. To reduce cross contamination, seedlings were grouped

by fungal treatments in the glasshouse, until ready for transplant. They were irrigated daily with deionized water and fertilized at 60, 80 and 100 days after transplanting with ammonium sulfate (0.4 g l^{-1} foliar application). To control *Perileucoptera coffeella,* seedlings were sprayed twice with Ethion 50 CE at 20-day intervals. Seedlings were maintained in the glasshouse for 4 months (to six pairs of leaves) before field transplanting.

Field planting and growth

Before transplanting, a sample of 10 seedlings was taken from each fungus treatment for growth and colonization assessments. AM colonization, assessed by the grid intersect method (Giovannetti and Mosse 1980), was 35% for seedlings inoculated with CM and less than 20% for all *G. etunicatum* isolates. Seedlings with CM and *G. etunicatum*-Var were slightly larger and had a higher leaf area and higher P content than those inoculated with other fungi or not inoculated. Four-month-old seedlings (outplants) were taken into the field plots in Patrocínio (MG) in January 1989 (rainy season) and planted according to the seedling and soil treatments previously described. After transplanting, seedlings were normally fertilized annually by soil application of 100 g plant $^{-1}$ N and 120 g plant $^{-1}$ K_2O and by four foliar applications of Zn (0.6% $ZnSO_4$), B (0.3% H_3BO_3), Cu (0.3% $CuCl_2$) and K (0.3% KCl). There were also two spray applications of Ethion 50 CE or Decis 25 CE for plant pest protection.

Measurements and statistics

Plant development after transplantation was assessed by measuring plant height, stem and canopy diameters, and survival at different time intervals. Root colonization, spore number in the soil, and leaf nutrient status were also assessed. For AM colonization and spore numbers, rhizosphere samples (0–20 cm depth) were collected at every plant and pooled per plot. Analyses were performed as previously mentioned. The nutrient content of leaf samples was determined after nitro-perchloric digestion according to Sarruge and Haag (1974). After ripening, coffee beans were harvested, allowed to dry under sunlight, and productivity recorded annually for 5 consecutive years. Yield data are reported as kg ha $^{-1}$ of unshelled beans.

The data were subjected to analysis of variance for P rates, mycorrhizal treatment and interactions using the statistical package SANEST (Instituto Agronômico de Campinas, SP). When a significant ($P \leq 0.05$) treatment effect was found, the mean values were compared using the Duncan tests for yield data and the Tukey test for all other variables, both at the 0.05 level. Regression analyses were performed for P effects on the mean values of the five P rates using statistical package SAEG (Universidade Federal de Viçosa, MG). The best-fit regression ($P \leq 0.05$) was selected and used to show the responses.

Table 1 Analysis of variance of coffee plant development in the field following pre-colonization with AM fungi and P application at planting in January 1989. Error degree of freedom = 102 (*NS* not significant)

	df	*F* value	$P \leq$
Plant height, October 89 (9 months)			
Phosphorus rate (P)	4	2.51	0.05
Fungal treatments (F)	6	12.58	0.01
Interaction (P × F)	24	1.06	NS
Block	3	1.90	NS
Plant height, August 90 (19 months)			
Phosphorus rate (P)	4	17.69	0.01
Fungal treatments (F)	6	3.42	0.01
Interaction (P × F)	24	0.98	NS
Block	3	3.00	0.05
Plant height, March (26 months)			
Phosphorus rate (P)	4	37.72	0.01
Fungal treatments (F)	6	2.06	NS
Interaction (P × F)	24	0.97	NS
Block	3	5.50	0.01
Canopy diameter, October 89			
Phosphorus rate (P)	4	5.62	0.01
Fungal treatments (F)	6	6.67	0.01
Interaction (P × F)	24	1.14	NS
Block	3	0.54	NS
Canopy diameter, August 90			
Phosphorus rate (P)	4	19.97	0.01
Fungal treatments (F)	6	3.28	0.01
Interaction (P × F)	24	1.02	NS
Block	3	0.54	NS
Canopy diameter, March 91			
Phosphorus rate (P)	4	62.34	0.01
Fungal treatments (F)	6	2.76	0.01
Interaction (P × F)	24	1.26	NS
Block	3	19.17	0.01
Stem diameter, October 89			
Phosphorus rate (P)	4	4.90	0.01
Fungal treatments (F)	6	8.00	0.01
Interaction (P × F)	24	1.22	NS
Block	3	0.32	NS
Stem diameter, August 90			
Phosphorus rate (P)	4	33.18	0.01
Fungal treatments (F)	6	3.38	0.01
Interaction (P × F)	24	0.99	NS
Block	3	1.26	NS
Stem diameter, March 91			
Phosphorus rate (P)	4	83.26	0.01
Fungal treatment (F)	6	1.31	NS
Interaction (P × F)	24	0.89	NS
Block	3	2.49	NS
Nodes per plant, August 90			
Phosphorus rate (P)	4	3.28	0.05
Fungal treatments (F)	6	3.38	0.01
Interaction (P × F)	24	1.22	NS
Block	3	0.12	NS

Results

Development of outplants in the field was highly influenced by both superphosphate (P) rates and fungal inoculation (Table 1). At 9 and 19 months after transplant (MAT), plant height, canopy, stem diameters were influenced by both factors, which did not interact. At a later stage, in March 1991 (26 MAT), only plant height and stem diameter were affected by P rates, whereas canopy diameter was affected by both factors, which did not interact. Phosphorus application was highly beneficial for plant development as measured by height and canopy and stem diameters, and fungus treatments also had significant effects on plant development (Table 2). In 1989 (9 MAT), all pre-colonized seedlings were taller (25–50% over control) than those without pre-colonization, whereas canopy diameter was only enhanced by *G. etunicatum* from Par, Pat, Var and Lav, which did not differ from one another. Mycorrhizal effects on plant height, as percent of control, were 50, 18 and 10% at 9, 19 and 26 MAT, respectively, i.e. diminished with time after transplanting. No significant effects on plant height and stem diameter were found at 26 MAT. Initial differences in plant development amongst outplants colonized by CM and *G. etunicatum*-Var were not detected after 19 MAT. This was the re-

Table 2 Development of pre-colonized or non-inoculated (Ni) coffee outplants in the field at 9, 19 and 26 months after transplanting into soil containing increasing P rates (g plant $^{-1}$ P_2O_5). Means followed by the same letter in each column are not significantly different by Tukey's test at P 0.05 (*CM* a mixture of *Glomus clarum* and *Gigaspora margarita, Var TP Lav Par Pat* different isolates of *Glomus etunicatum*)

		Plant height (cm)				Stem diameter (cm)			Canopy diameter (cm)		
		0	9	19	26	9	19	26	9	19	26
Fungus	Ni	10c	32c	76b	106a	5.5c	22.7b	24.1a	22c	71b	103b
	CM	15ab	42b	82ab	111a	7.4ab	24.8ab	24.4a	26bc	76ab	108ab
	TP	13bc	40b	81ab	109a	6.0bc	24.4ab	24.9a	25bc	77ab	106ab
	Par	11bc	43ab	88a	116a	7.5ab	26.7a	26.5a	27ab	85ab	111ab
	Pat	17a	43ab	86a	114a	7.1b	26.6ab	25.2a	26ab	85ab	116ab
	Var	13bc	43ab	87a	116a	8.2ab	27.0a	26.6a	30a	87a	117a
	Lav	13bc	48a	90a	117a	8.7a	27.6a	26.2a	30a	89a	115ab
P rate	0		39b	71c	90c	6.3b	18.9c	15.1d	23b	62c	79d
	20		42ab	83b	111b	7.5a	25.3b	25.4c	27a	80b	109c
	40		44a	88ab	118ab	7.7a	27.4ab	27.4bc	27a	88ab	116bc
	80		42ab	91a	121a	7.7a	29.1a	29.3ab	27a	91a	124ab
	160		41ab	89ab	121a	7.2ab	28.0ab	30.1a	26ab	88ab	125a

sult of colonization of non-inoculated plants by indigenous fungi, as discussed later. Canopy diameter of plants with *G. etunicatum*-Var differed from controls at 26 MAT and plants of this treatment were amongst the most productive, thus indicating pre-colonization effects. The number of nodes per plant, which indicates the length of yielding branches and yield potential, was also influenced by both factors but not by their interactions (Table 1). In contrast to fungus treatments, P rates were still highly effective on growth parameters at 26 MAT. Outplant survival was high and not affected by any treatment. Block effects on plant height at 19 and 26 MAT and on canopy diameter at 26 MAT were found (Table 1). Although sites appeared quite homogeneous and variability in growth over a site was expected to be minimal, the ANOVA indicates unwanted variability with time in the field. Other factors responsible for these effects can not be ruled out here, but it appears that blocking improved the capacity of the experiment to test treatment effects.

AM colonization at 26 MAT was high, ranging from 43 to 55%. It was not affected ($P \leq 0.05$) by pre-colonization but was reduced linearly by P rates (Fig. 1a). Spore numbers on the other hand, were low and were greatly depressed by P application (Fig. 1b). The number of spores of *G. margarita* ranged from 2 to 14 per 50 ml of soil, with the highest density found in the rhizosphere of CM plants. Spores of *G. etunicatum, G. clarum* and other species were recovered in very low numbers, indicating that pre-colonization of coffee outplants had little effect on spore populations in the rhizosphere at 26 MAT.

Leaf nutrient contents were influenced by both pre-colonization and by P application. Although most nutrients were affected, a consistent effect was only observed for P rates on foliar P. In 1990, leaf P content exhibited an 1.8-fold increase over the control with no P added (0.68 g kg $^{-1}$ leaf tissue) when 160 g plant $^{-1}$ P_2O_5 was applied at planting (1.23 g kg $^{-1}$); the fold-

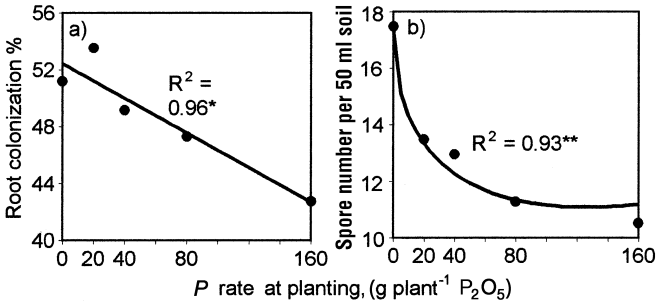

Fig. 1 Overall P effects on arbuscular mycorrhizal colonization and spore numbers in coffee outplants after 26 months in the field (**$P \leq 0.01$)

effect was reduced to 1.3 in the 1992 sampling (1.08 g kg $^{-1}$ control versus 1.23 g kg $^{-1}$ in high P). A mycorrhizal effect was found in 1992, when plants from most fungus treatments had higher P contents than controls. In 1991 and 1994, a significant ($P \leq 0.05$) interaction was found between P rates and fungus treatments (Table 3). Compared with the first sampling (1990), plants with no P added at planting increased leaf P content in 1994, from 0.68 to 1.02 g kg $^{-1}$, thus indicating a considerable change in plant ability to take up P under a limited supply of this nutrient in the soil. In 1991, only plants pre-colonized by *G. etunicatum*-Pat at the highest P rate had a higher P content than controls, whereas in 1994 no such differences were found (Table 3). CM plants showed reduced foliar P in 1991 at a P rate of 40 g plant $^{-1}$. This may result from mycorrhiza enhancement of early plant development, which may have affected P translocation to other parts of the plant. Leaf P content increased with increasing applied P at planting, but showed no correlation with yield, except in 1991 with leaf P in 1990 ($r = 0.47$*) and 1991 ($r = 0.57$*). In general, fungus treatments had only slight effects on leaf content of P and other nutrients. However, application of P favored uptake of Ca and Mg and reduced K

Table 3 Treatment effects on foliar P content (g kg $^{-1}$) in 1991 and 1994. Means followed by the same letter within in each row are not significantly different by Tukey's test at P 0.05 (Q quadratic, SR square root, L linear, other abbreviations as in Table 2)

P rates (g plant $^{-1}$)	Fungus treatment						
	Ni	CM	TP	Par	Pat	Var	Lav
1991							
0	0.065a	0.088a	0.093a	0.085a	0.093a	0.085a	0.078a
20	0.090a	0.110a	0.105a	0.123a	0.120a	0.123a	0.100a
40	0.125a	0.075b	0.123a	0.120a	0.125a	0.113a	0.110a
80	0.130abc	0.100c	0.148ab	0.135abc	0.125abc	0.150ab	0.128abc
160	0.108bc	0.128abc	0.140ab	0.135ab	0.145a	0.138ab	0.118abc
Regression	Q	no fit	Q	SR	L	Q	Q
R^2	0.94**		0.98**	0.96**	0.76**	0.85**	0.99**
1994							
0	0.102ab	0.114ab	0.012ab	0.097b	0.125a	0.106ab	0.116ab
20	0.113ab	0.124ab	0.126a	0.122a	0.114ab	0.130a	0.132a
40	0.121ab	0.115ab	0.116ab	0.129a	0.099b	0.110ab	0.127a
80	0.095a	0.116a	0.115a	0.114a	0.106a	0.108a	0.112a
100	0.109abc	0.126ab	0.129a	0.128ab	0.095c	0.102bc	0.102bc
Regression	no fit	no fit	no fit	SR	SR	no fit	SR
R^2				0.68**	0.85**		0.86**

** $P \leq 0.01$

(data not shown). Micronutrient contents were equally affected by both factors, but such effects were not consistently related to treatments, except for Zn and Cu which were reduced by P application. No major changes in other nutrients were found.

Coffee bean yield measured for 5 consecutive years, showing consistent effects of P application throughout the experiment, whereas significant ($P \leq 0.05$) mycorrhizal effects were only found in 1991 and 1994, when a P/mycorrhizal interaction was also found. At the first harvest (1991), all pre-colonized outplants gave a better yield than those without pre-colonization, with the responses affected by P applied at planting (Fig. 2). Maximal estimated yield was obtained with Par followed by Pat and Var, with yields in the range of 800 kg ha $^{-1}$, as compared with 580 kg ha $^{-1}$ for non-inoculated controls. This corresponds to a yield increase of 38%. In addition, plants raised from pre-colonized seedlings exhibited lower external P requeriments, achieving maximal yield with much less added P than those without pre-colonization. Based upon the response curves, P equivalent effects of mycorrhizal pre-colonization ranged from 65 to 103 g plant $^{-1}$ P_2O_5, with an average of 89 g plant $^{-1}$ or 254 kg ha $^{-1}$ P_2O_5.

At the second, third and fifth harvests (1992, 1993 and 1995) bean yield was higher (Table 4) than in 1991 as a result of crop development. Yield was greatly enhanced by P application but not by mycorrhiza. Phosphorus had a 6.58-, 8.17- and 4.72-fold increase over controls in 1992, 1993 and 1995, respectively. In 1994, an interaction was found between the two factors (Table 4). Plants from all treatments responded to P application. Significant mycorrhiza effects over non-inoculated plants were found in CM at 20 g plant $^{-1}$ P_2O_5 and *G. etunicatum*-Var and Lav at 160 g plant $^{-1}$ P_2O_5. CM plants also gave higher yields than any other pre-colonized outplants at the lowest P rate. At P rates of 80 g plant $^{-1}$ P_2O_5, *G. etunicatum*-Par gave a lower yield than non-inoculated plants. At this harvest, maximal yield was obtained with plants pre-colonized by *G. etunicatum*-Var at the highest P rate. This result was sig-

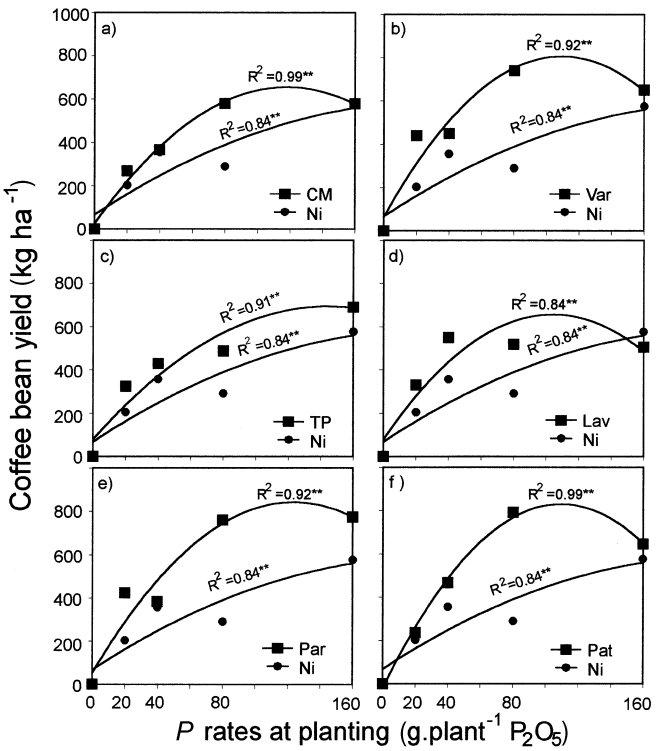

Fig. 2 Coffee bean yield at first harvest (1991) in response to P fertilization and AM pre-colonization. Outplants were pre-colonized with a mixture of *Glomus clarum* and *Gigaspora margarita* (CM) or with isolates of *Glomus etunicatum* (Var, TP, Lav, Par, Pat) or were non-inoculated (Ni). Best fit line at $P \leq 0.01$ shown

Table 4 Coffee yield (kg ha $^{-1}$) in 4 successive years (1992–95) as affected by pre-colonization with different AM fungi and P rates at planting (g plant $^{-1}$ P_2O_5). Means followed by the same letter (lower case within columns and capitals within rows for 1994 data) are not significantly different by Duncan's test at P 0.05. Abbreviations as in Table 2

P rate	1992	1993	1995	1994 (M × P interaction)						
				Ni	CM	Pat	Lav	Var	TP	Par
0	150c	167e	229c	63cA	15cA	147bA	45cA	12cA	5bA	47bA
20	598b	482d	386c	334bcB	1017abA	264bB	346bcB	267bcB	419abB	353aB
40	671b	741c	769b	815bcA	620bcA	705bA	790bcA	510bcA	446abA	718aA
80	859a	1144b	1071a	1645aA	1534aA	1468aAB	949bAB	885bAB	1228aAB	696aB
160	987a	1365a	1082a	941abC	1178abBC	718bC	1870aAB	2178aA	883aC	741aC

nificantly higher than non-inoculated and plants pre-colonized by several other isolates of *G. etunicatum.* Thus coffee bean yields obtained after the first harvest were highly inconsistent (Fig. 3). This is particularly evident in plants with Pat, CM and non-inoculated, which conformed to the known biennal "alternate yield cycle" of this crop under full sunlight (Wellman 1961) as in Brazil. It is interesting to note the steady increase in yield of non-pre-colonized plants up to the fourth harvest. In order to reduce the effect of this phenomenon, coffee researchers usually look at yield cycles or overall accumulated yields. Two- and 3-year cycles were not useful for assessing mycorrhizal effects in our study, while accumulated yield during the 5 harvests showed great and consistent effects of P rate and small effects of pre-colonization (Table 5). Non-inoculated

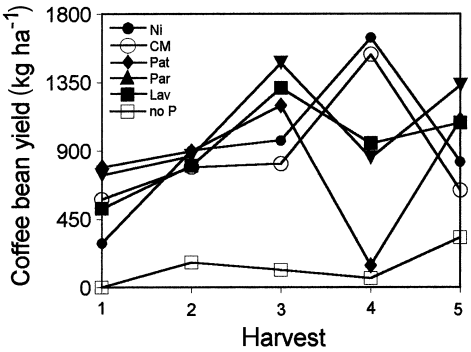

Fig. 3 Mean bean yield for coffee at five consecutive harvests (1991–1995) for plants not fertilized with P at planting (no P), or pre-colonized with mixture of *G. clarum* and *G. margarita* (*CM*) or isolates of *G. etunicatum* (*Pat, Var, Lav*) or non-inoculated (Ni), all the latter in the presence of 80 g plant $^{-1}$ P_2O_5

Table 5 Accumulated coffee yield (total of five harvests in kg ha $^{-1}$) as affected by mycorrhizal status of the outplants and P rate (g plant $^{-1}$) at planting. Means followed by the same letter (lower case within columns and capitals within rows) are not significantly different by Duncan's test at P 0.05. Abbreviations as in Table 2

P rate	Pre-colonization treatments						
	Ni	CM	Var	TP	Lav	Par	Pat
0	673dA	872cA	542cA	324cA	462cA	582cA	695cA
20	2158cAB	3500bA	2161bAB	1989bAB	1591cB	2249bAB	1865cB
40	3710bA	3062bA	3193bA	2879bA	3762bA	2944bA	3361bA
80	4636abA	4364bA	5299aA	4910aA	4668abA	4661aA	5461abA
160	5263aAB	5971aA	6006aA	4725aAB	5404aAB	5365aAB	4244aB

plants exhibited increasing yield to moderate P rates (20–80), whereas most pre-colonized plants showed no such differences, thus indicating the benefits of mycorrhiza. Pre-colonized plants did not differ ($P \leq 0.05$) from non-pre-colonized ones. However, at 20 g plant $^{-1}$ P_2O_5, CM plants gave a higher yield than those pre-colonized by two isolates of *G. etunicatum* (Lav and Pat). Although not significantly more ($P \leq 0.05$) than non-precolonized plants, CM plants produced 62% more coffee in the five harvests when fertilized with low P at planting. Additionally, at the highest P, CM and Var plants were more productive than those with Pat. In spite of some inconsistency amongst mycorrhizal treatments, pre-colonization effects remained detectable throughout advanced crop stages, 6 years after planting into the field. The complexity of P versus mycorrhizal interaction and the alternate yield cycle of coffee are very evident in the present study. This may account for the diminished and inconsistent beneficial effects of pre-colonization of coffee outplants over the 6-year period.

Discussion

When pre-colonized coffee seedlings were taken into a low-fertility field soil, early plant development and bean yield were favored, when compared to seedlings without pre-colonization in the nursery. For the first 2 years following transplantation, all measured parame-

ters were also highly influenced by the P rate applied at planting, and strong interactions between P rate and pre-colonization treatments were found. Pre-colonization had no effect when plants received no P at planting and mycorrhizal effects were maximal at moderate P rates. Phosphorus responses for the first harvest agreed with that reported for mycorrhizal coffee grown in a controlled enviromnent (Saggin-Júnior et al. 1994; Siqueira and Colozzi-Filho 1986), thereby confirming mycorrhizal nutritional benefits and the strong interrelationship between P supply and mycorrhizal response under nutrient-stressed conditions (Siqueira and Saggin-Júnior 1995). As previously reported for another field study in this soil, mycorrhizal benefit was only observed in the presence of applied P (Siqueira et al. 1993). In contrast to results for temperate conditions (Johnson and Pfleger 1992), in the highly weathered, acid tropical soils, where phosphorus is easily immobilized, mycorrhizal inoculation and soluble P were complementary. Therefore, they should be managed to enhance productivity in the early stages of coffee crops in low-fertility soils.

As demonstrated here and in a further study with coffee (Siqueira et al. 1993), the consistent effects of mycorrhiza on plant development and yield diminished or disappeared with time in the field. For example, mycorrhizal effects on external P requirement for maximal yield were high and consistent (found in all fungal treatments) at the first harvest but diminished and varied unpredictably with crop age. This is in accordance with Guimarães (1986), who showed that P required for maximal yield in a similar soil type diminished from 120 g plant $^{-1}$ P_2O_5 at the first harvest to 60 g plant $^{-1}$ P_2O_5 at the fourth harvest. This is a very complicated scenario considering the nature of the biological and chemical factors involved in such responses. Usually, young perennial plants exhibit a high external P requirement which diminishes as the plant ages. Coffee is not an exception to this generalization considering the low amount of P extracted by an adult crop (Mehlich 1967) and the fact that P is not a major limiting factor to coffee productivity of mature crops, when compared to other nutrients (Gallo et al. 1970). Increased root mass and its uptake activity and the establishment of mycorrhizal symbiosis with crop development in the field may contribute to low P requirements in developed coffee plants. This appears to be why it is recommended to apply a large quantity of P at planting but only maintainence P after crop establishment (Guimarães 1986). However, heavy application of soluble P at planting may reduce mycorrhiza formation, fungus sporulation, and the crop's mycorrhizal dependency, and thus mycorrhizal effectiveness for the developing crop. Results presented here for root colonization are in agreement with those found in the glasshouse (Saggin-Júnior et al. 1992) and the field (unpublished data) showing that non-mycorrhizal coffee seedlings readily became mycorrhizal when transplanted to AM fungus infested soil, i.e. in most agricultural fields. The ability of coffee plants to become mycorrhizal may also contribute to reduced pre-colonization effects for developed plants, especially when effective fungi are present in the indigenous fungal population. Therefore, the success of AM technology will depend upon finding well-adapted and superior fungal strains.

The alternate yield cycle of coffee in the field is another complicating factor in understanding P × mycorrhizal interactions and their effects on crop productivity. Plants with high yields in a given year are known to yield poorly at succeeding harvests. The physiological basis for this phenomenon is not completely known (Wellman 1961), but yield cycles are affected by nutrition and crop management practices. In fact, they were differently affected by treatments in this study (Fig. 3) and certainly contributed to the kind of responses and interactions we report for the 6-year period. In spite of the differences between some mycorrhizal and non-mycorrhizal outplants, all mycorrhizal outplants gave higher yields at the first harvest and highly inconsistent yields in succeeding ones, except for plants pre-colonized by CM and Var. These latter plants were slightly bigger than controls at planting and were the only ones to show differences in accumulated yield, thus indicating their better performance. Because AM colonization rates, growth parameters and yield at the second and third harvests were equal to those of non-mycorrhizal plants, higher accumulated yields can not be attributed to size differences at outplant stage but rather to fungal effects. Higher yield of CM and Var plants may result from direct fungal effects or fungal-phosphate interactions on successive harvests. Moreover, the fact that these plants were more productive than those with *G. etunicatum*-Pat (originally isolated from the experimental site) suggests that the mixture CM and *G. etunicatum*-Var is very promising for inoculation of coffee in this soil. Although not significant at $P \leq 0.05$, seedlings pre-colonized with the mixture of *G. clarum* and *G. margarita* at 20 g plant $^{-1}$ P_2O_5, appeared to give a yield increment of 1342 kg ha $^{-1}$. This corresponds to an additional gross income of US$ 5000 (March 1997) per ha of crop during the five harvest, thereby confirming the economical benefit of AM fungi. The cost of AM inoculation is not available because there is no commercial inoculum on the Brazilian market. However, based on an estimated cost of US$ 5.00 per thousand seedlings (Castellano and Molina 1989), mycorrhizal inoculation would not exceed US $ 20.00 per ha of coffee.

In conclusion, P application was essential for early crop development, coffee productivity and mycorrhizal benefits in a low-fertility soil in Brazil. Pre-colonization of coffee outplants with all AM fungi tested was highly effective in promoting yield at the first harvest, but gave reduced and inconsistent results in subsequent ones. Differential responses during the experiment appear to be related to reduction in external P requirement by the crop and to mycorrhizal colonization by indigenous AM fungi. Mycorrhizal effects on the first harvest were estimated to be equal to 254 kg ha $^{-1}$

P_2O_5. Because mycorrhizal plants were more productive at an early crop stage and showed no reduced yield in the developed crop when compared to those without pre-colonization, mycorrhiza technology represents a considerable saving on fertilizer for coffee agriculture in Brazil. Thus, pre-colonization of coffee outplants with selected AM fungi and application of low to moderate P rates at planting is highly advantageous for coffee production in low-fertility soils in Brazil.

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References

Castellano MA, Molina R (1989) The biological component: nursery pests and mycorrhizae. In: Landis TD, Tinus RW, McDonald SE, Barnett JP (eds) The container tree nursery manual. USDA, Washington, pp 101–167

Colozzi-Filho A, Siqueira JO, Saggin-Júnior OJ, Guimarães PTG, Oliveira E (1994) Efetividade de diferentes fungos micorrízicos arbusculares na formação de mudas, crescimento pós-transplante e produção do cafeeiro. Pesqui Agropecu Bras 29:1397–1406

Fernandes AB, Siqueira JO (1989) Micorrizas vesicular-arbuscular em cafeeiro da região sul do Estado de Minas Gerais. Pesqui Agropecu Bras 24:1489–1498

Gallo JR, Hiroce R, Bataglia OC, Moraes FRP (1970) Levantamento de cafezais do Estado de São Paulo, pela análise química foliar. II. Solos podzolizados de Lins e Marília, Latossolo Roxo e Podzólico Vermelho-Amarelo-Orto. Bragantia 29:237–247

Gerdemann JW (1968) Vesicular-arbuscular mycorrhizae and plant growth. Annu Rev Phytopathol 6:397–418

Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans Br Mycol Soc 46:235–244

Giovannetti M, Mosse B (1980) An evaluation of techniques to measure vesicular-arbuscular mycorrhizal infection in roots. New Phytol 84:489–500

Guimarães PTG (1986) Respostas do cafeeiro (*Coffea arabica* L. cv CATUAI) à adubação mineral e orgânica em solos de baixa fertilidade do sul de Minas Gerais. PhD thesis, ESALQ-USP, Piracicaba

Janse JM (1897) Les endophytes radicaux de quelques plantes javanaises. Ann Jard Bot Buitenzorg 24:53–201

Johnson NC, Pfleger FL (1992) Vesicular-arbuscular mycorrhiza and cultural stresses. In: Bethenfalvay GJ, Linderman RG (eds) Mycorrhizae in sustainable agriculture. American Society of Agriculture, pp 71–79

Lopes ES, Toledo SV, Hiroce R, Dias R, Oliveira E (1983) Efeitos do fungo micorrízico *Gigaspora margarita* no desenvolvimento e aproveitamento do fósforo e do zinco em mudas de cafeeiro "Mundo Novo" em casa de vegetação. In: Congresso de Pesquisas Cafeeiras, IBC/GERCA, pp 121–122

Malavolta E, Kliemann HJ (1985) Desordens nutricionais no cerrado. Potafos, Piracicaba

Mehlich A (1967) Mineral nutrition in relation to yield and quality of Kenya coffee. Effect of nitrogen fertilizers, mulch and other materials on yield grade "A" coffee. Kenya Coffee 32:399–407

Minas Gerais-Secretaria do Estado da Agricultura Pecuária e Abastecimento (1995) Cenário futuro do negócio agrícola de Minas Gerais, Belo Horizonte, vol VII

Saggin-Júnior OJ, Siqueira JO (1995) Avaliação da deficiência simbiótica de fungos endomicorrízicos para o cafeeiro. Rev Bras Ci Solo 19:221–228

Saggin-Júnior OJ, Siqueira JO, Colozzi-Filho A, Oliveira E (1992) A infestação do solo com fungos micorrízicos no crescimento pós-transplante de mudas de cafeeiro não micorrizadas. Rev Bras Ci Solo 16:39–46

Saggin-Júnior OJ, Siqueira JO, Guimarães PTG, Oliveira E (1994) Interação fungos micorrízicos versus superfosfato e seus efeitos no crescimento e teores de nutrientes do cafeeiro em solo não fumigado. Rev Bras Ci Solo 18:27–36

Saggin-Júnior OJ, Siqueira JO, Guimarães PTG, Oliveira E (1995) Colonização micorrízica do cafeeiro por diferentes fungos micorrízicos: Efeitos na formação das mudas e no crescimento em solo fumigado. Rev Bras Ci Solo 19:213–220

Sarruge JR, Haag HP (1974) Análises químicas em plantas. ESALQ-USP, Piracicaba

Sieverding E (1991) Vesicular-arbuscular mycorrhiza management in tropical agrosystems. Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn

Siqueira JO, Colozzi-Filho A (1986) Micorrizas vesículo-arbusculares em mudas de cafeeiro. II. Efeito do fósforo no estabelecimento e funcionamento da simbiose. Rev Bras Ci Solo 10:207–211

Siqueira JO, Saggin-Júnior OJ (1995) The importance of mycorrhizal association in natural low fertility soils. Machado (eds) International Symposium on Environmental Stress: Maize in Perspective. EMBRAPA/CIMMYT, pp 240–280

Siqueira JO, Colozzi-Filho A, Saggin-Júnior OJ, Guimarães PTG, Oliveira E (1993) Crescimento de mudas e produção do cafeeiro sob influência de fungos micorrízicos e superfosfato. Rev Bras Ci Solo 19:53–60

Siqueira JO, Saggin-Júnor OJ, Flores-Aylas WW (1995) Occurrence and diversity of the endomycorrhizal Glomalean fungi in coffee fields in southeastern Brazil. In: International Symposium on Microbial Ecology. International Committee on Microbial Ecology/Brazilian Society for Microbiology, pp 72

Wellman FL (1961) Coffee: botany, cultivation and utilization. Hill, London