Effects of Vesicular-Arbuscular Mycorrhizal Fungi on the Mixed Cropping of Maize and Beans[1]

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ABSTRACT

A study was carried out under greenhouse condition to determine the effect of four mycorrhizal fungus species (genus *Glomus*), all native of Viçosa, State of Minas Gerais, on beans (*Phaseolus vulgaris* L.) cultivated alone and in association with maize. The four species had a similar behavior in relation to maize and bean root colonization. When beans were cultivated in association with maize, the four native mycorrhizal species increased their production. No such effect was observed in monocropped beans, because the soil utilized had good fertility. When mycorrhizal root fragments were used as an inoculant, maize was a better host than bean for fungus multiplication. In another study carried out in the field, in which maize was cultivated in association with beans, *Glomus etunicatum* only increased the maize production. The maximum distance reached by this fungus, when spreading through soil, was 50 cm, 64 days after plant emergence.

RESUMO

Em condições de casa de vegetação, foi estudado o efeito de quatro espécies defungos micorrízicos do gênero *Glomus*, nativas da região de Viçosa, Estado de Minas Gerais, sobre o cultivo consorciado de milho e fiejão (*Phaseolus vulgaris* L.) e sobre o monocultivo do feijão. Todas as espécies comportaram-se de modo semelhante ao infectar as raízes das duas culturas. As quatro espécies micorrízicas nativas aumentaram a produção do feijoeiro, quando ele foi cultivado em associação com o milho. Isso não ocorreu quando a leguminosa ficou isolada. O milho mostrou-se melhor hospedeiro para multiplicação de fungos micorrízicos, atuando melhor nas inoculações por intermédio de raízes infectadas. No consórcio milho-feijão, no campo, *Glomus etunicatum* proporcionou aumento de produção apenas do milho. Esta espécie de fungo dispersou-se pelo solo, até a distância máxima de 50 cm, no período de 64 dias após a emergência das plantas.

INTRODUCTION

Interplanting maize (*Zea mays*) and common bean (*P. vulgaris*) is a common practice among small farmers in Minas Gerais and other Brazilian states. It is well known that both crops, when planted alone, have a growth response when inoculated with vesicular-arbuscular mycorrhizal (VAM) fungi. However, no information was found in the literature on the effects of mycorrhizal fungi on maize and beans when grown together. The purpose of this study was to investigate such effects.

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MATERIALS AND METHODS

Five species of VAN fungi of the genus *Glomus* were tested. Four, identified here as *Glomus* 1, *Glomus* 2 (both collected from bean rootlets in the field), *Glomus* 3, and *Glomus* 4 (collected from maize rootlets, in the field) are native to the Viçosa area. The fifth species, *G. etunicatum* Becker & Gerd., was introduced from the University of Florida.

The bean cultivar Milionário 1732 and the maize cultivar Cargill 111 were used in this study.

First experiment

The experiment was carried out under greenhouse conditions, in pots containing 3 kg of clay soil previously passed through a 5 mm mesh sieve and then treated with methyl bromide (100 cm^3/m^3). Prior to planting, the pots were fertilized with triple superphosphate (150 ppm of P) and potassium chloride (80 ppm of K) and the soil pH corrected to 6.0 with dolomitic limestone. Seven days after plant emergence, a micronutrient solution containing H_3BO_3 (1.86 g/l), $MnCl_2.4H_2O$ (5.28 g/l), $ZnSO_4.7H_2O$ (7.04 g/l), $CuSO_4.5H_2)$ (2.09 g/l), $NaMoO_4.2H_2O$ (0.09 g/l), $FeCl_3.6H_2O$ (3.01 g/l) was applied at a rate of 8 ml per pot. Thirty days after plant emergence, urea (30 ppm of N) was applied as top-dressing on pots containing only bean plants. However, on pots with maize and maize-bean mixture, urea was added 30, 40, and 50 days after plant emergence, at the rate of 30 ppm of N per application.

The experimental design was a 4 x 2 x 2 factorial with 4 VAM fungal treatments (*Glomus* 1, *Glomus* 2, *Glomus* 3, and *Glomus* 4), two cultural treatments (maize and bean in the same pot versus separate pots), and two inoculation treatments (inoculated versus uninoculated pots). Each inoculated pot received 1000 spores placed 7 cm below the surface. Five replicate pots were used for each treatment. Two to there seeds were planted per pot, and subsequently thinned to one plant five days after emergence. The pots containing the cereal-legume mixture were thinned to one plant of each species. To evaluate infection incidence, an auger was used to collect root samples down to 10 cm at 30 and 60 days after emergence. Root colonization was estimated according to the method of Giovannetti and Mosse (5) using 10% KOH cleared roots stained with 0.1% lactophenol trypan blue (15). Root segments were considered colonized if either hyphae, arbuscules, vesicles, or spores were visible. Seed productions were determined only for beans.

Second experiment

This experiment was performed under greenhouse conditions and its objective was to evaluate the infection incidence using infected root fragments as the inoculum. To obtain the inoculation material, maize roots previously inoculated with *Glomus* 3 or *Glomus* 4 were collected 30 and 60 days after plant emergence. The roots were washed, cut into small pieces and placed 7 cm below the soil surface in pots containing 3 kg of soil previously treated with methyl bromide and fertilized as in the first experiment. Afterward, two to three bean seeds were planted in each pot and later thinned to one plant.

The same procedure was employed for bean roots inoculated with *Glomus* 1 and *Glomus* 2. They were washed, cut into small pieces and placed in pots where maize was planted. Each of the eight treatments was replicated five times. Infection was evaluated 45 days after plant emergence.

In the first and second experiments, powdery mildew (*Erysiphe polygoni* DC ex Merat) and tropical mites (*Polyphagotarsonemus latus* (Banks)) attacked the bean plants. Effective control was achieved with Dinocap (0.5 g/l) applied 25 and 35 days after plant emergence.

Third experiment

This experiment was carried out under field conditions. The soil was inoculated with 20 000 spores per meter of *G. etunicatum* applied at a depth of seven centimeters. To distribute the spores evenly throughout the furrow, the inoculum was first mixed with soil (250 ml/m).

The experimental design was a randomized complete block with three planting treatments: maize and beans interplanted in the same row, maize and beans in separate rows 50 cm apart. There were two inoculation treatments, inoculated and uninoculated furrows, for each planting treatment. Each experimental unit was 1 m in length and replicated four times.

Only the rows containing maize were inoculated with VAM fungus. Plots were overseeded and hand-thinned to five maize or 14 bean plants per meter for the single-crop rows and five maize plus 14 beans per meter for the mixed-croprows.

The soil was treated with methyl bromide (100 cm^3/m^3). The plots were fertilized with urea, triple superphosphate, and potassium chloride at a rate of 60 (N) - 160 (P_2O_5) - 90 (K_2O) kg/ha. The fertilizer was broadcasted over the plots and incorporated into the soil by harrowing. Thirty days after plant emergence, 30 kg/ha of N (ammonium sulfate) was applied as top-dressing. To avoid water stress, plots were irrigated to 10% of yield capacity.

For root colonization evaluation, samples were taken with an auger 15 cm below the soil surface at 34, 49 and 64 days after plant emergence. When maize and beans were planted in the same row, two root samples were taken per plot, at a distance of 3 cm from the inoculation point. When the crops were planted in separated rows, fours samples were taken per plot, two at 3 cm from the inoculation point and two at 3 cm from the bean stems.

A final sampling was performed at 78 days after plant emergence, at 10, 20, 30, and 40 cm from the inoculation point. For yield determination, 3 maize plants and 14 bean plants per plot were harvested and weighted.

RESULTS AND DISCUSSION

First experiment

Table 1 shows bean yields for the 16 treatment combinations. The effects of inoculation and cultural methods were both significant at the 0.01 level. The interaction of cultural method x VAM species was also significant, at the 0.05 level. Yields were significantly higher in the pots containing only beans. This is likely due to the competitive effects of maize, which can reduce yields of an interplanted crop 50% to 70% (17).

Table 1. Effect of native species of *Glomus* on yield of beans planted alone or jointly with maize.

VAN fungi species	Cultural method	Inoculation	Yield (g)	Yield increase (%)
Glomus 1	Only bean	Inoculated	10.48	+ 10.6
		Uninoculated	9.48	
	Bean + Maize	Inoculated	5.08	+ 62.8
		Uninoculated	3.12	
Glomus 2	Only bean	Inoculated	11.10	+ 11.9
		Uninoculated	9.92	
	Bean + Maize	Inoculated	3.50	+ 16.7
		Uninoculated	3.00	
Glomus 3	Only Bean	Inoculated	9.60	+ 1.0
		Uninoculated	9.50	
	Bean + Maize	Inoculated	4.18	+ 16.1
		Uninoculated	3.60	
Glomus 4	Only Bean	Inoculated	11.54	- 1.4
		Uninoculated	11.70	
	Bean + Maize	Inoculated	4.22	+ 27.9
		Uninoculated	3.30	

When grown alone, bean yield was about 10% higher in the presence of *Glomus* 1 and *Glomus* 2, the species collected from bean rootlets at the field. *Glomus* 3 and *Glomus* 4, the species collected from maize rootlets, had no effect on bean yield.

When beans were associated with maize in the same pot, inoculation significantly increased bean yield across all native species of *Glomus*. Therefore, when bean and maize were grown together and both colonized with VAN fungi, the competitive ability of the legume was enhanced. It seems that, under conditions of competition for P and other elements uptake from the soil, as occurs in associated cropping, mycorrhizal fungi can improve the yield ability of legumes.

It is likely that the four *Glomus* species would promote yield increases of the bean cultivated alone, if the soil available P content had been lower. Ross (16) has observed, in *Glomus* inoculated soybeans, yield increases of 122%, 67% and 12%, when the level of the applied P was low, medium and high, respectively. The high P content of the soil (107 ppm) probably did nor permit the full expression of VAM fungi potential.

In this experiment, maize yield was not measured and only percentage of colonized root was determined. Initially, only small differences in colonization incidence occurred among the mycorrhizal species, *Glomus* 1 and *Glomus* 2 presented an average of 36% colonization in maize, 36 days after plant emergence. *Glomus* 3 and *Glomus* 4 presented an average of 29% colonization incidence higher than 95%. In beans, 30 days after emergence the average colonization incidence was 33%, with *Glomus* 1 and *Glomus* 2, and 24% with *Glomus* 3 and *Glomus* 4; but 60 days after emergence, it was higher than 97%. When both plants were grown together, *Glomus* 1 and *Glomus* 2 caused an average colonization incidence of 34%, while *Glomus* 3 and *Glomus* 4 caused 31%, 30 days after plant emergence; however, 60 days after emergence, the colonization incidence surpassed 95% for the four species.

Second experiment

Results on mycorrhizal fungi spread from one crop to another are on Table 2. Infected roots of both beam and maize were able to transmit the mycorrhizal fungi to the other species. When cultivated over bean roots infected with *Glomus* 1 and 2, the average infection incidence on maize roots reached 73.6%. This was practically twice as much (37.9%) as that found in bean roots grown over maize roots infected with *Glomus* 3 and 4. The difference occurred in spite of the similar infection percentages of both crops roots used as inoculum. This demonstrated that maize is a better host than beans for mycorrhizal fungi multiplication.

When maize colonization is well established and bean colonization is initiated, a nutrient transfer from maize to bean plant can be expected, at least initially. Hypha interconnections between roots of the same or different plant species had already been proved to take place (7, 8).

Table 2. Mycorrhizal colonization incidence of maize and bean at 45 days, grown in pots inoculated with *Glomus* species from bean and maize roots.

Crop	Inoculation source	Mycorrhizal species[1/]	Days after plant emergence[2/]	Colonization incidence at 45 days (%)
Maize	Bean roots	*Glomus* 1	30	72.3
			60	87.6
		Glomus 2	30	63.0
			60	71.4
Bean	Maize roots	*Glomus* 3	30	34.9
		Glomus 4	30	31.7
			60	42.9

1/ *Glomus* 1 and 2 were isolated from bean roots; *Glomus* 3 and 4 were isolated from maize roots.

2/ When inoculated roots used as inoculum were collected.

In the field, bean plants can benefit from hypha interconnections if planted within 10 cm from the maize plants. According to Whittingham and Read (21), nutrient transfer among plants is, ecologically, a very important phenomenon, since young plant survival depends upon such transfer when they grow among wellestablished plants that exploit the available nutrients intensively. The access, through hyphae of VAM fungi, to phosphorus reserves from neighbor plants is, therefore, of considerable benefit to plants at the initial stage of growth.

In the Zona de Mata area, State of Minas Gerais, the yield of dry season beans, when in relay cropping with maize, is sometimes higher than the yield of beans alone, when rains are scarce (18). It is likely that this benefit from associated cropping can be explained, at least in part, by the effect of VAM fungi that develop at maize roots and quickly pass to young bean roots. In this way, bean roots would be quickly colonized by mycorrhizal fungi, improving P and water absorption.

Third experiment

Results for second experiment (Table 3) show the percentages of root colonization. The rows containing maize all had similar results: at 34 days after plant emergence, colonization was already high (> 40%). Colonization percentage in beans planted 25 cm from inoculated maize had a low colonization percentage, even at 64 days after emergence. The colonization percentage when beans were 50 cm from the inoculated maize was even lower: less than 2% colonization in bean rootlets at 64 days after plant emergence. Those results demonstrated that *G. etunicatum* will have little effect on bean plants when the inoculum is 25 to 50 cm away. At this distance, the colonization index was very low and, in addition, the colonization took place when bean plants had already passed the critical growth stage (60 days after planting) when mycorrhizal fungi are important.

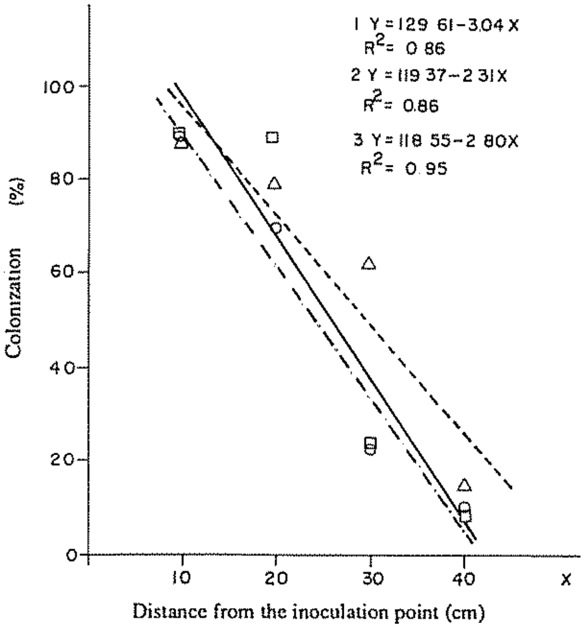
Table 3. *G. etunicatum* percentage colonization of maize and bean roots.

Treatments	Crops	Days after plant emergence 34	49	64
Maize with beans in the same row, inoculated[1/]	Maize and bean[2/]	45.3	79.4	99.4
Inoculated maize with bean 25 cm distant	Maize	42.0	76.3	99.4
	Bean	0.0	5.9	8.7
Inoculated maize with bean 50 cm distant	Maize	47.1	89.0	99.5
	Bean	0.0	0.0	1.6

1/ 20 000 spores per meter of row

2/ Mixed maize and bean roots.

The movement of *G. etunicatum* from the inoculation point at 78 days after plant emergence is shown in Figure 1. When beans were planted jointly with maize, colonization rates at the distances of 10 and 20 cm from the inoculation point were similar, but decreased considerably at distances greater than 20 cm. When beans were planted 25 cm away from the inoculated maize, colonization at the distance of 30 cm from the inoculation point was higher than in the other two bean plant arrangements. It is likely that this occurred because, at that distance, bean root number, and consequently root volume, was very high; therefore, a better condition for VAM development would be present. When beans were planted 50 cm distant from the inoculated maize, the colonization rate was the lowest.



1. □ ......maize and beans in the same row
2. Δ .... maize and beans in different rows at a 25 cm interval.
3. O ..... maize and beans in different rows at a 50 cm interval.

Fig. 1 *G. etunicatum* spread in three bean-plant arrangements in association with inoculated maize, 78 days after plant emergence

It was found, therefore, that colonization rates at different distances from the inoculated maize varied according to bean plant arrangements, but were almost the same at 40 cm from the inoculation point. Warner and Mosse (19) found that for *Trifolium repens* and *Festuca rubra*, in sole croppings, root density is important for *G. fasciculatum* spread, but the species effect was much more important. In *T. repens*, a greater root density was favorable for fungus colonization, which did not occurr in *F. rubra*.

According to results presented by several authors, spread of mycorrhizal fungi in the soil is highly variable, depending on the interaction between soil-plant-fungus. Reports of mycorrhizal spread from the inoculation point vary from as little as 5 cm after 15 weeks (19) to distances greater than 4.5 m after 15 weeks (12).

The difference in the ability of mycorrhizal fungi to spread can also be attributed to their capacity to reach, through their external hyphae, different distances from the root surface. There are reports that those fungi can spread both small distances from the root surface (20) and long distances, up to 16 cm (23).

In this study, *G. etunicatum* spread through soil at a maximum distance of 25 and 50 cm, 49 and 64 days after plant emergence, respectively; this can be attributed to the influence of irrigation water. The study was carried out during the dry season and thus daily irrigations (with sprinklers) were necessary. According to Mosee *et al.* (12), the transfer of a small amount of soil carrying VAM fungi spores by wind, rain, and irrigation water cannot be excluded under field conditions. McIlveen and Cole (11) also attribute to soil movement the dissemination of mycorrhizal fungi in the field.

Maize yields were significantly higher at the 0.05 level for the inoculated treatments versus uninoculated treatments across all planting treatments (Table 4). Inoculation with VAM fungus resulted in a 19-21% yield increase for the maize in mixed cropping. As widely reported (2, 3, 4, 6, 9, 10, 13), mycorrhizal association is advantageous for maize, but all the mentioned studies were carried out with maize in monoculture.

Table 4. Maize yield with and without inoculation with *G. etunicatum*, in different types of association with beans.

Treatments		Yield (g)	Production increase caused by inoculation (%)
Maize and beans in the same row	Inoculated[1/]	708 00[2/]	21.08
	Uninoculated	584 75	
Maize with beans 25 cm distant	Inoculated[1/]	769 53[2/]	19.44
	Uninoculated	644 25	
Maize with beans 50 cm distant	Inoculated[1/]	707 75[2/]	19.45
	Uninoculated	592 50	

1/ 20 000 spores per meter of row
2/ Indicates significant difference at the 0.05 level between the inoculated and uninoculated treatments.

Table 5. Bean yield when planted in the same row with maize, inoculated or not with *G. etunicatum*.

	Yield[1/] (g)	Yield increase due to inoculation (%)
Inoculated	74.2	2.48
Uninoculated	72.4	

1/ 14 plants per plot. The yield difference is not significant at 0.05 level (t test)

Bean yield (Table 5) was determined only for the planting treatment with maize and beans in the same row. Although it has been reported that *G. etunicatum* is effective for increasing bean growth and yield (22), this study, with beans interplanted with maize, showed no significant effect of fungal inoculation. Parada (14) mentions that any alteration in the soil-fungus-plant system can modify the relationship among them. The presence of maize roots near the bean roots may have affected the mycorrhizal fungus-bean root interaction. Another possible reason for the ineffectiveness of fungal inoculation of bean plants is that the maize plant density was too high. The recommended plant population for mixed cropping of maize and beans should not exceed 40 000 maize plants per hectare. Denser populations are disadvantageous for bean yield, because maize is a competitive plant (1, 17). In this study, the maize density was 5 plants per meter or 50 000 per hectare. It is likely, therefore, that the maize competition nullified any beneficial effect of *G. etunicatum* on the bean growth and yield, since the roots of the latter crop presented a high percentage of colonization by the fungus. In addition, the bean plants were not subjected to water stress and low P fertilization, two conditions that favor VAM fungi effect.

CONCLUSIONS

1. Native mycorrhizal species had a similar behavior in relation to maize and bean root colonization, but the species *Glomus* 1 and *Glomus* 2 were slightly more aggressive for both crops.

2. When beans were cultivated in association with maize, the four native mycorrhizal species increased their production; however, no such effect was observed on monocropped beans, because the soil used had adequate fertilization.

3. When mycorrhizal root fragments were used as inoculant, maize was a better host than bean for the VAM fungus multiplication.

4. *G. etunicatum* was effective in increasing maize yield across all planting treatments, but not effective in increasing bean yields.

5. Maximum distance reached by *G. etunicatum*, when spreading through soil, was 25 and 50 cm from the inoculation point, 49 and 64 days after plant emergence, respectively.

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