Effectiveness of Arbuscular Mycorrhizal Fungal Isolates from the Land Uses of Amazon Region in Symbiosis with Cowpea

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ABSTRACT

Arbuscular mycorrhizal fungi provide several ecosystem services, including increase in plant growth and nutrition. The occurrence, richness, and structure of arbuscular mycorrhizal fungi communities are influenced by human activities, which may affect the functional benefits of these components of the soil biota. In this study, 13 arbuscular mycorrhizal fungi isolates originating from soils with different land uses in the Alto Solimões-Amazon region were evaluated regarding their effect on growth, nutrition, and cowpea yield in controlled conditions using two soils. Comparisons with reference isolates and a mixture of isolates were also performed. Fungal isolates exhibited a wide variability associated with colonization, sporulation, production of aboveground biomass, nitrogen and phosphorus uptake, and grain yield, indicating high functional diversity within and among fungal species. A generalized effect of isolates in promoting phosphorus uptake, increase in biomass, and cowpea yield was observed in both soils. The isolates of *Glomus* were the most efficient and are promising isolates for practical inoculation programs. No relationship was found between the origin of fungal isolate (*i.e.* land use) and their symbiotic performance in cowpea.

Key words: biodiversity, Inceptisol, Oxisol, root colonization, *Vigna unguiculata*.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF - Phylum Glomeromycota) are important components of terrestrial ecosystems where they have a pivotal role in providing several ecosystem services (Gianinazzi et al. 2010). The mycorrhizal colonization of the root cortex and the external mycelia produced in the soil by AMF act as access routes for plants to uptake low-mobility nutrients, including phosphorus (P), resulting in increased nutrient absorption and often plant growth (Smith and Read 2008). Similar to other components of the soil biota, changes in land use can modify AMF community structure and richness (Stürmer and Siqueira 2011) and consequently compromise the benefits of AMF to these communities.

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Although AMF have no host specificity, these fungi can exhibit different degrees of symbiotic compatibility with host plants, which are affected by host and fungal genotype (Van der Heijden et al. 1998, Smith et al. 2000), and environmental factors (Koide 1991). Furthermore, large functional variability among distinct AMF species and fungal isolates (=lineages) of the same species (Klironomos 2003, Smith et al. 2000, 2004) have been demonstrated. Munkvold et al. (2004) argue that intraspecific variations are of great ecological importance, particularly in situations with low species diversity, considering that a high intraspecific functional diversity could compensate for the low species diversity with regard to the symbiotic effectiveness of AMF. However, these variations are poorly known, particularly considering AMF isolates originating from tropical soils.

Thonar et al. (2011) observed distinct spatial efficiencies in P uptake by *Rhizophagus intraradices*, *Claroideoglomus claroideum*, and *Gigaspora margarita*, which help to explain the empirical evidence that root colonization by multiple AMF species may be more beneficial to plants than colonization by a single species (Jansa et al. 2008). However, there is evidence that most of the benefits to host plants could be achieved using a single species, provided that the species is efficient and adapted (Daft and Hogarth 1983, Edathil et al. 1996). Therefore, a high taxonomic diversity of AMF species in a particular system would not necessarily result in increased benefits to the host plants. Another important aspect is related to the interaction of different isolates with their host species. Santos et al. (2008) observed varying degrees of effectiveness of AMF isolates, depending on the host species evaluated, and observed that mixtures of isolates were the most efficient fungal treatments. Fungal isolates pertaining to different species from the same mycorrhizal community exhibited distinct efficiencies in promoting the

accumulation of leaf biomass and phosphorus content in soybean and red clover, but at least one isolate of each community was efficient in promoting plant growth and nutrition (Stürmer 2004).

Despite the geographic extent and the high plant species richness detected in the Amazon tropical forest, only recently the composition of AMF species from distinct land use systems has been accessed (Leal et al. 2009, 2013, Stürmer and Siqueira 2011, Freitas et al. 2014) and on the functional diversity of AMF communities (Silva et al. 2009). Amazon soils exhibit high richness of AMF species, and 61 morphotypes have been reported to occur in soils under different land uses in the Western Amazon region, of which 30% represent undescribed species (Stürmer and Siqueira 2011). Studies on the inter and intra-specific variation about the effectiveness of AMF to promote plant growth have been done mainly with fungal isolates from temperate countries under temperate conditions (Munkvold et al. 2004, Koch et al. 2004, Antunes et al. 2011, Mensah et al. 2015). In tropical region, Siqueira et al. (1998) found high intra-specific variation on effectiveness among isolates of *Claroideoglomus etunicatum* when inoculated in coffee plants.

Fungal communities originating from distinct land use system in the Brazilian Amazon region screened for their effectiveness had contrasting effectiveness in promoting plant growth and nutrition of cowpea, indicating the presence of efficient fungal lineages within each community that can be selected for inoculation programs (Silva et al. 2009). In this study, fungal isolates belonging to distinct genera and species established in single cultures from these communities were compared according to their effectiveness in two types of soils. Cowpea was selected as the host plant because of its importance as cash and food crop in the Amazon region and other tropical countries. The following hypothesis were tested: 1) difference

in effectiveness is related to the taxonomic position and origin (land use) of the fungal isolates, and 2) isolate effectiveness varies according to soil type.

MATERIALS AND METHODS

The study was conducted in a greenhouse in two sequential stages, each comprising two soil types with different physical and chemical characteristics. Stage 1 was conducted for 120 days in order to evaluate grain yield, mycorrhizal root colonization and AMF spore number. Once plants of stage 1 were harvested, cowpea seeds were sown into the same containers with the same treatments to start stage 2 in order to evaluate nodulation by rhizobia, plant growth (shoot dry matter) and nutrition (P and N content and accumulation) and mycorrhizal root colonization. Stage 2 was required for the following reasons: 1) logistic problem to sample and transport large amounts of the "Amazon soil", as this soil came from the western Amazon region, about 5,000 km from where the experiments were conducted, and 2) evaluation of cowpea yield is at the end of the plant growth cycle when the plant is completely dried, precluding the evaluation of nodulation and shoot nutrients, that are commonly evaluated at the flowering stage.

The soil designated "Amazon soil" was a typical haplic, aluminous Inceptisol with a clayey texture, collected from a depth of 0-20 cm in a pasture land covered with imperial grass. This area was originally covered by equatorial evergreen forest and is located in the city of Benjamin Constant, Amazonas state, Brazil. Amazon soil physical and chemical characteristics are described by Moreira et al. (2009). After the incubation period with dolomitic limestone to raise the base saturation, the Amazon soil was fumigated with methyl bromide (98% methyl bromide + 2% chloropicrin) at a dose of 393 $cm^3.m^{-3}$ After liming, soil contained the following chemical characteristics: pH in water=6.2; P content=4.9 $mg.dm^{-3}$ (Mehlich-1); K^+

content=86 $mg.dm^{-3}$ (Mehlich-1); Ca^{+2} content=7.8 $cmol_c.dm^{-3}$; Mg^{+2} content=3.9 $cmol_c.dm^{-3}$; Al^{+3} content=0.0 $cmol_c.dm^{-3}$; H+Al content=2.6 $cmol_c.dm^{-3}$; V=82.1%, and OM=3.3 $dag.kg^{-1}$. The soil designated "Lavras soil" was a substrate composed of sterile sand mixed with a red-yellow latosol (Oxisol) (1:2, vol/vol) with a clayey texture, collected from the 0-20 cm layer from a native forest located on the campus of the Universidade Federal de Lavras/UFLA, in Lavras, Minas Gerais state, Brazil. Chemical characteristics after soil liming were: pH in water = 5.9; P = 4.9 mg dm^{-3} (Mehlich-1); K^+ = 50 mg dm^{-3} (Mehlich -1); Ca^{2+} = 1.7 $cmol_c$ dm^{-3}; Mg^{2+} = 0.9 $cmol_c$ dm^{-3}; Al^{3+} = 0.0 $cmol_c$ dm^{-3}; H + Al = 2.6 $cmol_c$ dm^{-3}; V = 51.2 % and OM = 2.1 $dag kg^{-1}$. After liming and fumigation, both soils were transferred to 3.0 dm^3 plastic containers.

The experimental design incorporated randomized blocks with 2 plants per container and 5 replicates. The study comprised 23 fungal treatments: 13 isolates from different land uses in the Amazon region, 3 reference isolates obtained from the culture collection of UFLA, and 6 artificial communities made up by mixing fungal isolates from the same land use, and 1 non-inoculated control (Table I). *Glomus* sp11 and *Glomus* sp14 could not be identified to species level. *Glomus* sp11 forms spores with 63 μm diameter with a spore wall formed by an outer sloughing layer and a laminated layer formed by one lamina 0.6 μm thick, resembling *Glomus minutum*. *Glomus* sp14 differentiates a spore wall formed by an evanescent outer layer up to 2 μm thick and a finely laminated layer 6-7 μm thick. Spore color is cream yellow and subtending hypha near the spore has laminated layer 2μm thick which extends down to the hyphae and gets thinner, resembling *G. versiforme*. Reference isolates were single cultures of *Claroideoglomus etunicatum* (Ce), *Rhizophagus clarus* (Rc), and *Gigaspora margarita* (Gm). The 6 artificial communities

TABLE I
List of the AMF fungal species and isolates used for the efficiency experiment with cowpea.

Identification code	Species
Ad-F*	*Acaulospora delicata*
Ad-CV	*Acaulospora delicata*
Am-CV	*Acaulospora morrowiae*
G11-CV	*Glomus* sp. 11
G11-CN	*Glomus* sp. 11
G14-CN	*Glomus* sp. 14
Ad-AF	*Acaulospora delicata*
G11-AF	*Glomus* sp. 11
G14-AF	*Glomus* sp. 14
Am-R	*Acaulospora morrowiae*
Ad-R	*Acaulospora delicata*
G11-P	*Glomus* sp.11
Ad-P	*Acaulospora delicata*
Ce	*Claroideoglomus etunicatum*
Rc	*Rhizophagus clarus*
Gm	*Gigaspora margarita*
M-CN **	*Glomus* sp.11 + *Glomus* sp.14
M-CV	*Acaulospora delicata* + *Acaulospora morrowiae* + *Glomus* sp. 11
M-AF	*Acaulospora delicata* + *Glomus* sp. 11 + *Glomus* sp. 14
M-R	*Acaulospora delicata* + *Acaulospora morrowiae*
M-P	*Glomus* sp. 11 + *Acaulospora delicata*
MT	Ad-F-*Acaulospora delicata* + G11-P-*Glomus* sp. 11 + G14-CN-*Glomus* sp.14+ Am-R-*Acaulospora morrowiae*

*F - Forest; CV - *Capoeira Velha* (old secondary forest); CN - *Capoeira Nova* (Young secondary forest); AF - Agroforest; R - crop; P - Pasture; **All artificial communities (M) were supplemented with single cultures of: *Claroideoglomus etunicatus* (Ce), *Rhizophagus clarus* (Rc) and *Gigaspora margarita* (Gm); MT - Isolates mixtures with the diverse species from Amazon region.

were supplemented with a mixture of the reference isolates. AMF spores recovered from trap cultures established with soil from distinct land use systems with *Sorghum sudanense* and *Vigna unguiculata* as hosts (Leal et al. 2009) were extracted by wet sieving following a centrifugation step using water and a sucrose solution of 500 g.L^{-1} (Gerdemann and Nicolson 1963) and used to establish single cultures. Stocks of these cultures were later propagated under greenhouse conditions at UFLA using pueraria (*Pueraria phaseoloides*) and brachiaria (*Brachiaria decumbens*) as hosts for 150 days.

Cowpea (*Vigna unguiculata* L. cv. BRS 17 Gurgueia) is a variety resistant to the cowpea golden mosaic virus (CpGMV) and was provided by Embrapa Meio Norte, located in Teresina, Piauí, Brazil. Seeds were pre-germinated on filter paper in germination chambers. At the moment that seedlings were transferred to containers filled with 3.0 dm^3 of each soil, they were inoculated with a suspension of AMF spores (approximately 250 spores per container) for each inoculation treatment, in addition to the non-inoculated controls (NI). For the artificial communities treatment, the total number of spores was equally divided by the number of AMF species comprising this inoculum. All treatments were inoculated with 1 mL of *Bradyrhizobium sp.*, strain INPA03-11B containing

10^9 viable rhizobial cells mL^{-1}. To standardize the microbiota between the inoculated treatments and NI, 10 mL of a filtered inoculum without AMF spores was added to each container of the control group. Twenty days after transferring the pre-germinated seedlings to the containers, 20 mL of a Hoagland and Arnon (1950) solution without P or N, was applied every 20 days. The nutrient mixture applied per container contained 14.0 mg of potassium, 5.8 mg of magnesium, 9.53 mg of calcium, 20.9 mg of sulphur, 61.0 µg of boron, and 1.28 µg of molybdenum. Irrigation was performed to maintain the soil moisture at 60% of the total pore volume (TPV) filled with water.

After 120 days of growth, between November 2007 and March 2008 (stage 1), plants were removed from the containers and separated into shoots and roots. After washing the roots, approximately 1.0 g of fresh roots was removed from each plant for clearing and staining (Phillips and Hayman 1970) and subsequent evaluation of mycorrhizal colonization (Giovannetti and Mosse 1980). The pods from each plant were air dried. The plant seeds were harvested when the pods were ripe (yellow colour) and the grain yield per container was determined after drying in a forced air oven at 60 ºC until constant weight. Spore extraction was performed by wet sieving and sucrose centrifugation as described above and counted under a dissecting microscope at 20x to 40x magnification to determine the spore density per 50 mL of soil for all the AMF-inoculated treatments.

Stage 2 was conducted for 85 days (April to July 2008) until flowering after harvesting plants of stage 1. Plants were inoculated with *Bradyrhizobium* sp., and received the same amount of the nutrient solution without N and P as for stage 1. The total nutrient mixture applied per container contained 9.33 mg of potassium, 3.85 mg of magnesium, 6.41 mg of calcium, 14.00 mg of sulphur, 40.67 µg of boron, and 0.85 µg of molybdenum. Irrigation was performed to maintain the soil moisture at 60% of the total pore volume (TPV) filled with water.

At harvest, plants were removed from the containers, and shoots separated from roots. After washing the roots, the number and weight of nodules were determined, and approximately 1.0 g of fresh roots was removed from each plant for assessment of mycorrhizal colonization as in stage 1. The plant material was dried in an oven with forced air circulation at 60 °C until a constant weight was achieved, and shoot dry mass (SDM) was determined. The SDM was ground and chemically analysed to determine the levels of N and P, according to Zarosky and Burau (1977).

Data from the stages 1 and 2 were subjected to analysis of variance and means were separated using the Scott-Knott test, at a 5% confidence interval using the Sisvar statistical software (Ferreira 2011). Values of mycorrhizal colonization, nodulation, and sporulation previously transformed via arc sin $(x/100)^{1/2}$, $(x+1)^{1/2}$, and log $(1+x)$, respectively.

RESULTS

ROOT COLONIZATION AND SPORULATION

Considering stages 1 and 2 of the experiment, mycorrhizal colonization of cowpea ranged from 1% to 82%, according to the fungal treatment, with an overall mean of 32% and 33% for the Amazon and Lavras soils, respectively (Table II). In the Lavras and Amazon soil, 60% and 45% of the inoculated plants, respectively, exhibited a mycorrhizal colonization higher than 20%. No evidence of colonization was observed in plants of the NI treatment in either soil type or stage. Among the fungal treatments, only the Ad-F isolate did not colonize roots in either of the two stages in the Amazon soil, whereas the root colonization by this isolate was >10% in the Lavras soil. In the Amazon soil, at stage 2, the Am-CV and G11-CV isolates did not colonize the plant roots and did not differ from the Ad-F, Ad-CV, G14-AF, Am-R, and Ad-R

TABLE II
Mycorrhizal colonization of cowpea 120 days after inoculation (DAI - stage 1) with diverse treatments of inoculation of arbuscular mycorrhizal fungi (AMF) and 85 days after harvest of stage 1and new planting (stage 2), and spores number 120 DAI - stage 1, in Amazon and Lavras soils.

Treatments (AMF isolates)*	Colonization stage 1		Colonization stage 2		Spores number stage 1	
	Amazon soil	Lavras soil	Amazon soil	Lavras soil	Amazon soil	Lavras soil
	%				50 mL of soil	
Ad-F	0 d	10 c	0 d	3 d	219 b	3,170 b
Ad-CV	5 d	4 c	1 d	6 d	709 a	1,538 c
Am-CV	2 d	4 c	0 d	4 d	511 a	703 d
G11-CV	1 d	39 a	0 d	22 c	610 a	3,018 b
G11-CN	47 b	71 a	54 b	42 b	30 c	8,510 a
G14-CN	26 c	28 b	60 b	76 a	379 a	1,580 c
Ad-AF	16 c	14 b	10 c	25 c	2,899 a	10,645 a
G11-AF	5 d	3 c	8 c	6 d	40 c	3,376 b
G14-AF	18 c	26 b	2 d	20 c	762 a	6,290 a
Am-R	4 d	11 c	4 d	5 d	171 b	1,480 c
Ad-R	7 d	10 c	6 d	14 c	524 a	982 d
G11-P	39 b	58 a	13 c	25 c	109 b	2,916 b
Ad-P	61 a	15 b	16 c	16 c	257 b	4,479 b
Ce	11 c	17 b	7 c	16 c	200 b	2,949 b
Rc	74 a	69 a	76 a	41 b	160 b	261 e
Gm	7 d	52 a	6 c	43 b	138 b	591 d
M-CN	44 b	49 a	76 a	76 a	174 b	1,800 b
M-CV	60 a	66 a	77 a	46 b	409 a	3,263 b
M-AF	49 b	53 a	82 a	44 b	162 b	4,465 b
M-R	59 a	63 a	72 a	32 b	262 b	2,798 b
M-P	65 a	59 a	69 a	55 b	253 b	4,780 b
MT	74 a	55 a	77 a	60 a	314 a	3,211 b
NI	-	-	-	-	-	-

*See table I for identification and origin. Means followed by the same letter belong to the same group by Scott-Knott test at 5%.

isolates, which yielded a low level of colonization in this soil type. Mycorrhizal colonization by the G11-CN and G14-CN isolates from *capoeira nova* (young secondary forest) was intermediate to high in the second stage of the experiment, ranging from 54-60% in the Lavras soil and from 42-76% in the Amazon soil. However, the colonization level did not differ significantly between these fungal treatments compared to the isolate mixtures. *Acaulospora delicata* Ad-P was the only isolate that produced a high level of colonization in the Amazon soil, reaching 61% in stage 1. However, this fungus colonized only 16% of roots in stage

2. Colonization by isolate G11-CV, from *capoeira velha* (old secondary forest), was not significantly different from that of the Ad-F isolate in the Amazon soil but achieved a value of 22-39% in the Lavras soil.

The mycorrhizal colonization by the fungal isolates from the UFLA collection tended to be higher in the Lavras soil than in the Amazon soil, with the exception of the Rc isolate, which reached a colonization level of 74-76% at both of the stages of the experiment in the Amazon soil (Table II). High levels of colonization were obtained with

inoculation of artificial communities: 67% in Amazon soil and 55% in Lavras soil.

The sporulation by fungal isolates differed between both soil types (Table II). In Amazon soil, sporulation ranged from 30 to 2,899 spores in 50 mL of soil (mean = 422 spores), whereas in Lavras soil, sporulation varied from 261 to 10,645 spores in 50 mL of soil (mean = 3,309 spores). Of the five isolates of *A. delicata*, Ad-CV, Ad-AF, and Ad-R isolates sporulated abundantly in the Amazon soil, whereas Ad-F and Ad-P had lower levels of sporulation. The Ad-AF isolate from agroforests produced the greatest number of spores in both of the soil types, ranging from 2,899 to 10,645 spores in 50 mL of soil. Of the *Glomus* sp.11 isolates, only the G11-CV from *capoeira velha* achieved a high spore numbers in Amazon soil (610 spores in 50 mL of soil). Isolates G11-CN and G11-AF from *capoeira nova* and agroforests, respectively, achieved low levels of sporulation, amounting to 30 and 40 spores in 50 mL of soil, respectively. *Acaulospora morrowiae* Am-CV isolate sporulated 67% more than *A. morrowiae* Am-R originated from farmlands in the Amazon soil. In general, sporulation tended to be higher in Lavras soil compared to Amazon soil. Isolate G11-CN in Lavras soil achieved a sporulation level of 8,510 spores in 50 mL of soil, which is approximately 284 times higher than the level found in the Amazon soil for the same isolate. Similarly, the sporulation of G11-AF in the Lavras soil was approximately 85 times greater than in the Amazon soil.

PLANT RESPONSES TO MYCORRHIZATION

Fungal treatment affected all variables assessed in stage 2 for both soil types. A significant effect on SDM production in Amazon soil was observed for all fungal treatments, and ranged from 233% to 622% increase compared to NI (Table III). Eighteen out of the 22 inoculation treatments yielded the highest production of SDM compared to NI, with an average increase of 511%. In the Amazon soil, all artificial communities were among the treatments with the greatest capacity to increase cowpea growth.

Variation in growth after fungal inoculation was larger in Lavras soil, and four distinct groups was observed in this soil type. Only Am-CV did not differ significantly from NI, and the growth increments of the remaining isolates ranged from 308% to 950% (Table III). In the Lavras soil, 8 out of 22 inoculation treatments produced the highest increase in SDM, with a mean increase of 792% compared to NI. Among these treatments, isolates Ad-F, Ad-P, and Gm promoted SDM best increase in Lavras soil in relation Amazon soil. The smallest effects on SDM production were observed with *A. delicata* isolates (Ad-CV and Ad-R), which achieved a mean growth increase of 366% compared to NI. The effect of the isolate mixtures in the Lavras soil was much lower than that observed in the Amazon soil. The fungal isolates that had the greatest effect on growth, regardless of the soil type, were the *Glomus* isolates from *capoeira nova* (G11-CN and G14-CN) and *Acaulospora* isolate from farmlands (Am-R) in addition to the communities MR and MT.

Number of nodules in cowpea differed between soil types and was reduced in Amazon soil (Table III), where nodulation was only observed in seven treatments with fungal isolates (G14-CN, G11-P, MCN, MCV, MR, MP, and MT). In Lavras soil, nodulation was widespread among the fungal treatments, with an overall mean of 21 nodules per container. Plants inoculated with Am-CV and Ad-R isolates produced very low number of nodules that were not significantly different from NI plants.

Grain yield was influenced by fungal treatments in both the soil types (Fig. 1). Only the Ad-F isolate, which did not colonize cowpea in the Amazon soil, did not promote significant grain yield (Fig. 1). Non-inoculated plants grew very little and did not produce grains in either soil type.

TABLE III
Shoot dry mass (SDM), nodules number (NN) and dry mass (NDM) of cowpea 85 days after harvest of stage 1, when diverse treatments of arbuscular mycorrhizal fungi were applied, and new planting (stage 2), in Amazon and Lavras soils.

Treatments*	SDM		NN		NDM	
	Amazon soil	Lavras soil	Amazon soil	Lavras soil	Amazon soil	Lavras soil
	g pot⁻¹		nodules pot⁻¹		mg pot⁻¹	
Ad-F	3.01 b	3.20 a	0.0 b	19.20 b	0.0 b	70.00 a
Ad-CV	4.85 a	1.99 c	0.0 b	9.20 c	0.0 b	30.00 b
Am-CV	5.49 a	0.85 d	0.0 b	2.20 d	0.0 b	0.00 c
G11-CV	4.70 a	2.63 b	0.0 b	13.00 b	0.0 b	50.00 b
G11-CN	5.22 a	3.56 a	0.0 b	26.20 a	0.0 b	90.00 a
G14-CN	4.48 a	3.35 a	11.20 a	9.40 c	50.00 a	40.00 b
Ad-AF	5.63 a	2.72 b	0.0 b	15.20 b	0.0 b	50.00 b
G11-AF	2.96 b	2.95 b	0.0 b	16.40 b	0.0 b	60.00 b
G14-AF	5.41 a	2.53 b	0.0 b	18.00 b	0.0 b	20.00 c
Am-R	5.69 a	3.99 a	0.0 b	27.00 a	0.0 b	110.00 a
Ad-R	4.76 a	1.55 c	0.0 b	5.00 d	0.0 b	10.00 c
G11-P	6.04 a	2.28 b	12.40 a	13.80 b	30.00 a	20.00 c
Ad-P	2.96 b	3.24 a	0.0 b	35.40 a	0.0 b	90.00 a
Ce	4.56 a	2.37 b	0.0 b	16.20 b	0.0 b	30.00 b
Rc	5.84 a	2.43 b	0.0 b	16.40 b	0.0 b	20.00 c
Gm	3.33 b	3.25 a	0.0 b	33.60 a	0.0 b	60.00 b
MCN	5.41 a	2.50 b	1.60 b	23.20 b	0.00 b	40.00 b
MCV	5.83 a	2.64 b	19.40 a	20.60 b	40.00 a	30.00 b
MAF	5.52 a	2.84 b	0.0 b	26.80 a	0.0 b	50.00 b
MR	6.43 a	3.34 a	3.60 b	45.60 a	10.00 b	130.00 a
MP	5.77 a	2.96 b	3.00 b	28.80 a	0.00 b	50.00 b
MT	6.37 a	3.16 a	22.20 a	35.40 a	50.00 a	90.00 a
NI	0.89 c	0.38 d	0.0 b	0.0 d	0.0 b	0.0 c

*See table I for identification and origin. Means followed by the same letter belong to the same group by Scott-Knott test at 5%.

Considering cowpea yield after 120 days of inoculation (stage 1), three classes of treatments is distinguished based on the grouping criteria after the Scott-Knott test (Fig. 1). High effectiveness treatments (bars with the letter "a") comprised 13 and 10 treatments in Amazon and Lavras soils, respectively, and included all artificial communities and the reference *Gigaspora margarita* (Gm) for both soils (Fig. 1). Isolates common to both soils in this group included *Glomus* G11-P, G14-AF, originated from Pasture and Agroforestry, respectively. Medium effectiveness treatments (bars with the letter "b") comprised most of the remaining single isolates (Fig. 1). *Rhizophagus clarus* (Rc) and *C. etunicatum* (Ce) were within the high effectiveness group in Amazon soil but at the medium effectiveness group in Lavras soil. Isolate *Acaulospora delicata* Ad-F was in the non-efficient treatment in Amazon soil but in the medium efficient treatment in Lavras soil (Fig. 1). Overall, 57% and 43% of the treatments in the Amazon and Lavras soils, respectively, were included in the high efficient treatment. On average, cowpea grain yield in this group increased by 65% compared to the medium efficient group in Amazon soil but only increased by 34% in Lavras soil. It was also
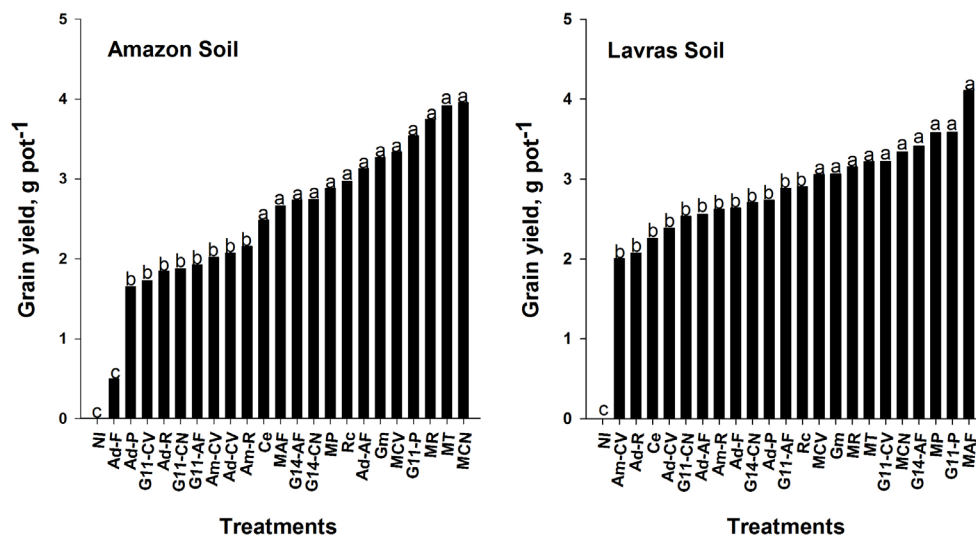
Figure 1 - Cowpea yields 120 day after inoculation (stage 1) with diverse treatments of arbuscular mycorrhizal fungi (see table I for AMF identification and origin), in Amazon and Lavras soils. Means followed by the same letter belong to the same group by Scott-Knott test at 5%.

observed a significant and positive correlation between colonization rate and grain yield in cowpea in both of the soils (r=0.58 in the Amazon soil, r=0.61 in the Lavras soil; both at $p \leq 0.01$).

CONTENTS AND ACCUMULATION OF N AND P IN COWPEA

There was a large variation in N content in SDM, from 17.92 to 58.52 g.kg^{-1}, and the largest content was found in the plants inoculated with MCN in the Amazon soil (Table IV). In the Lavras soil, the N contents in SDM were lower than in the Amazon soil, the highest N content in this soil of the Lavras, was obtained with isolate G11-CV. The benefits of AMF associated with P content were pronounced and widespread in the Amazon soil and were observed in all the treatments, with increases ranging from 33% to 149% compared with NI, and the mean P content was 1.15 g.kg^{-1}. The highest contents were found in the plants inoculated with MCV, MR, Rc, MP, and G14-AF, and the levels reached 1.52 g.kg^{-1}, two times higher than those found in the NI group. In the Lavras soil, because of the low production of SDM in the NI group, it

was not possible to determine the concentration of P in the control plants. In the inoculated plants, the mean P content was 0.90 g.kg^{-1}.

The accumulation of N and P were higher in all inoculated treatments in Amazon soil than in Lavras soil. The N accumulation in the Amazon soil varied from 120.59 to 314.05 mg of N per container, which was higher than that found in the Lavras soil (19.90 to 88.83 mg of N per container). In the Amazon soil, the largest P contents were observed in the plants inoculated with Rc, MCV, MR, and MP, with increases that ranged from 0.78 to 0.91 g.kg^{-1} compared to the NI group. In the Lavras soil, the highest accumulations of P in SDM were obtained in 68% of the treatments. In general, the accumulation of P in the Lavras soil was lower than that of the Amazon soil, with an average of 2.47 mg of P per container, which was two times lower than that recorded in the Amazon soil (5.78 mg of P per container).

DISCUSSION

Studies aiming to select efficient AMF isolates for inoculation according to particular soil

TABLE IV

Nitrogen (N) and Phosphorus (P) contents and accumulation in the shoot dry mass of cowpea 85 days after harvest of stage 1, when diverse arbuscular mycorrhizal fungi (see table I for identification and origin) were inoculated, and new planting (stage 2), in Amazon and Lavras soils.

Treatments	Nitrogen				Phosphorus			
	Amazon soil	Lavras soil	Amazon soil	Lavras soil	Amazon soil	Lavras soil	Amazon soil	Lavras soil
	g kg^{-1}		mg pot^{-1}		g kg^{-1}		mg pot^{-1}	
Ad-F	49.84 b	25.76 b	140.27 c	83.38 a	0.99 c	0.91 b	2.85 d	2.89 a
Ad-CV	39.20 c	23.52 c	187.06 b	48.84 c	0.91 d	1.05 a	4.43 c	2.11 b
Am-CV	36.12 d	23.80 c	197.48 b	19.90 d	0.91 d	0.92 b	4.99 c	0.78 c
G11-CV	40.88 c	33.88 a	184.58 b	88.83 a	1.09 c	1.01 a	5.08 c	2.65 a
G11-CN	39.76 c	18.48 c	204.35 b	65.21 b	1.09 c	0.91 b	5.77 b	3.23 a
G14-CN	33.32 d	21.00 c	144.43 c	69.51 b	1.17 c	0.81 c	5.32 c	2.73 a
Ad-AF	33.88 d	21.84 c	184.58 b	59.32 b	1.09 c	1.06 a	6.18 b	2.85 a
G11-AF	45.36 b	22.96 c	131.83 c	67.23 b	1.22 b	0.98 a	3.56 b	2.90 a
G14-AF	36.96 d	23.24 c	198.62 b	58.66 b	1.37 a	1.02 a	7.33 b	2.64 a
Am-R	35.00 d	21.84 c	198.27 b	87.09 a	1.01 c	0.94 b	5.73 b	3.77 a
Ad-R	39.48 c	18.76 c	184.04 b	29.72 d	1.09 c	0.95 b	5.27 c	1.46 c
G11-P	32.20 d	27.44 b	188.53 b	63.93 b	1.10 c	0.83 c	6.65 b	1.90 b
Ad-P	47.60 b	19.04 c	127.45 c	61.72 b	1.09 c	0.78 c	3.20 b	2.50 a
Ce	36.96 d	20.16 c	163.13 c	46.57 c	0.98 c	0.82 c	4.44 c	1.97 b
Rc	28.56 e	24.08 c	161.76 c	58.08 b	1.39 a	0.87 c	8.09 a	2.10 b
Gm	46.76 b	20.16 c	153.09 c	66.77 b	1.28 b	0.78 c	4.22 c	2.54 a
MCN	58.52 a	20.44 c	314.05 a	49.36 c	1.20 b	0.80 c	6.41 b	1.99 b
MCV	26.04 e	19.60 c	153.32 c	51.76 c	1.52 a	0.92 b	8.79 a	2.43 a
MAF	21.84 e	20.72 c	120.59 c	58.95 b	1.15 c	0.90 b	6.36 b	2.53 a
MR	34.72 d	17.92 c	220.09 b	59.33 b	1.44 a	0.91 b	9.25 a	3.04 a
MP	24.08 e	17.92 c	137.22 c	53.51 c	1.38 a	0.85 c	7.97 a	2.51 a
MT	26.32 e	21.00 c	168.35 c	65.83 b	0.81 d	0.90 b	5.18 c	2.83 a
NI	- *	- *	- *	- *	0.61 e	- **	0.40 e	- **

* and ** insufficient material or determination of nitrogen and phosphorus, respectively. Means followed by the same letter belong to the same group by Scott-Knott test at 5%.

conditions and crops involve culturing these fungi in single cultures and screening them for plant growth and nutrition promotion under controled conditions (Abbott and Robson 1982). These procedures were adopted in the present study by focusing in screening fungal isolates originated from communities previously tested for their effectiveness in promoting growth and nutrition of cowpea. Our results evidenced i) the presence of high efficient fungi originating from distinct land use types, suggesting that fungal origin is not a determinant of a fungus and effectiveness, and ii) effectiveness of a given fungal isolate was conserved among both soil types tests.

Mycorrhizal root colonization levels was stable within the same fungal isolate growing in both soil types. For example, root colonization of *Acaulospora delicata* (Ad) and *A. morrowiae* (Am) from *capoeira velha* (CV) was always ≤ 6% in both soil types. Furthermore, intraspecific variability in root colonization was observed in the present study: isolates from the same species (e.g., G11 and Ad)

exhibited low (<10%) to high (>25%) colonization rates. This result suggests that fungal growth within the root system may be determined by intrinsic factors of the fungal isolates (Munkvold et al. 2004, Koch et al. 2004). The range of mycorrhizal colonization in cowpea reported in the present study was quite high but was within the range previously observed for this species. Muthukumar and Udaiyan (2002) reported colonization rates of cowpea greater than 60%, whereas Rohyadi et al. (2004), under soil acid conditions (pH 4.7), reported a colonization rate of 35%. Among the reference isolates, *Gigaspora margarita* (Gm) yielded a low colonization rate in Amazon soil and a high colonization rate in Lavras soil, which may reflect local adaptation of this fungus to this soil condition as this isolate is originated from the same region where the Lavras soil was collected. A meta-analysis of studies published between 1988 and 2003 by Lekberg and Koide (2005) reported that an increase in mycorrhizal colonization resulted in an average increase of 23% in plant yield in the field. This change may be of particular importance in the Amazon region, where soils in deforested areas rapidly lose fertility and the strategy to maintain plant production to sustain local population is to advance deforestation to new areas. It is essential to find alternative strategies capable of enhancing plant nutrition and health, thereby ensuring plant production, reducing deforestation pressure and thus leading to the sustainability of agricultural systems. In this context, the identification of efficient AMF isolates capable of improving crop yield is of great interest to ensure food safety in the Amazon region.

The spore production by the AMF isolates in Lavras soil tended to be higher than that observed in the Amazon soil, indicating that chemical and physical soil characteristics impact fungal reproductive strategy via asexual spores in these fungi. The Amazon soil was clayey and very cohesive when dry and exhibited a low porosity,

which may have contributed to a lower sporulation level, considering that clayey soils tend to impact negatively upon AMF spore densities (Sieverding 1991). In the present study, the number of spores from isolates of the same species varied considerably depending on its origin and type of soil. For example, isolate G11-CN in the Lavras soil had a sporulation level 284 times greater than the level found in the Amazon soil, despite the origin of this fungus was from an Amazonian soil. Among the isolates of *Glomus* sp.11 and *A. morrowiae*, only the G11-CV and Am-CV isolates, both from *capoeira velha*, had a high sporulation level in the Amazon soil. These results suggest that AMF can rapidly undergo genotypic changes that influence their fitness (in this case, measured by the sporulation level), according to the environmental conditions in which they are cultivated (Angelard et al. 2014). Because of these changes, the effectiveness of an isolate tested under controlled conditions can change rapidly under field conditions, and this variability should be considered when choosing AMF isolates for selection programs.

Single (a single AMF isolate) and multiple (artificial communities) inoculation were able to stimulate the growth and yield of cowpea, although to different degrees. Am-CV was the only isolate that failed to promote growth in the Lavras soil compared to NI plants. Similarly, Ad-F was the only isolate that had no significant effect on grain yield in the Amazon soil, which can be explained by the absence of plant colonization by this isolate in this soil. This generalized response of cowpea to AMF has been well documented (Almeida et al. 1985, Rohyadi et al. 2004), and the beneficial effects of AMF are attributed mostly to the enhancement of plant nutrition (Koide 1991, Smith et al. 1992), especially considering P uptake from the soil. Some isolates, including G11-P, G11-CV, Ad-AF, G14-CN, G14-AF, and the artificial communities increased 2.5 to 3.0 times the grain yield of cowpea compared to NI plants. These results indicate a large

effectiveness of AMF originating from distinct land uses in the western Amazon soils and the presence of highly efficient isolates in mycorrhizal communities, which can be considered promising for future studies of the application of AMF to production systems in the Amazon region. Notably, the most promising isolates were originated from areas with different land uses, for example, G14-CN and G14-AF were isolated from areas occupied by *capoeira nova* and agroforests, respectively.

To determine the reasons for the poor nodulation in the Amazon soil, an additional test was conducted using unsterilized Amazon soil to verify whether the low nodulation level was associated with soil sterilization, considering that the clayey texture of this soil type may have contributed to a residual effect of the sterilizing agent. The nodule formation in sterilized and unsterilized soils followed the same pattern: a few small nodules, and most of the fungal treatments did not yield any nodulation (data not shown). Therefore, methyl bromide did not influence the formation of nodules in the samples evaluated. Moreover, the lower nodulation level in the Amazon soil in comparison with the Lavras soil might be derived from the soil characteristics—clayey texture, low porosity, and low water infiltration—resulting in reduced aeration, thus hindering the development of nodules and consequently reducing biological nitrogen fixation.

The content and accumulation of shoots N and P in differed markedly among fungal treatments. In Amazon soil, all the fungal treatments that produced the highest contents of N resulted in the lowest increases in SDM compared to NI, except to that was in plants inoculated with MCN, indicating a concentration effect for those treatments with lowest SDM and highest N content. The isolate of *R. clarus* and the artificial communities MCV, MAF, MP, and MT were the fungal treatments that yielded the lowest shoot N content of cowpea in the Amazon soil. This pattern most likely resulted from

the effect of dilution because all of these fungal treatments are among the treatments that promoted the highest production of SDM. This dilution effect on mycorrhized plants has been observed in soybeans (Paula et al. 1988), in various tree species (Flores-Aylas et al. 2003), and in cowpea (Silva et al. 2009).

The results indicate a large variability among the fungal isolates in the uptake of P, particularly in the Amazon soil, where 4 distinct groups were found. Although a direct relationship between the increase in SDM and P content from the soil can not be established, it appears that all the treatments in which inoculation increased SDM resulted in increased P levels in the plant, a fact that has been previously reported (Sanni 1976, Islan et al. 1980, Almeida et al. 1985, Rohyadi et al. 2004, Silva et al. 2009). Isolates efficient in absorbing P can reduce P deficits through increased absorption and increased P use by plants (Koide 1991) and by enhancing plant colonization by these fungi (Smith et al. 1992).

Inoculation with artificial communities, including MP, MR, and MCV, was most effective in increasing cowpea P levels and promoting growth and grain yield of cowpea in the Amazon soil. These results confirm to some extent those found by Santos et al. (2008), who used AMF isolates from bauxite-mining soils in the early growth of native plant species. These authors observed that isolate mixtures exhibited the greatest effectiveness in increasing plant growth compared to the non-inoculated control. However, in the present study, the isolates G11-CN, G14-CN, and Am-R also exerted strong effects on growth, irrespective of the growth conditions. These results corroborate those found by Daft and Hogarth (1983) and Edathil et al. (1996), indicating that the maximum benefits for growth can be achieved using a single and efficient AMF species and that isolate mixtures composed of a high diversity of species do not necessarily translate into increased benefits to plants.

The large inter- and intraspecific effectiveness of AMF tested in the present study is of great importance for controled inoculation programs in Amazon soils. Among the isolates tested, the most promising for cowpea were those from the genus *Glomus*. Considering that this genus is quite abundant, diverse, and adapts easily to different soil conditions, species of this genus should be prioritized when selecting fungal isolates to efficiently inoculate crops of interest in the Amazon region. Studies aimed at establishing the relationship between functional diversity and benefits to plants are essential and will help elucidate the relationship between biodiversity and ecosystem functioning. Considering the large extent and variation of Amazon ecosystems, this study contributes to the understanding of the functional diversity of AMF in the Alto Solimões region in the state of Amazonas and provides useful information for the exploitation of these important genetic resources in the Amazon biome.

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