ARBUSCULAR MYCORRHIZAL FUNGI SELECTION FOR *Coffea canephora* Pierre ex A. Froehner CLONAL CULTIVAR CONILON 'BRS OURO PRETO'

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(Recebido: 09 de novembro de 2016; aceito: 13 de março de 2017)

ABSTRACT: Coffee production in Brazil expands to warmer regions with *Coffea canephora* clonal cultivar Conilon 'BRS Ouro Preto'. The response of 'BRS Ouro Preto' to arbuscular mycorrhizal fungi (AMF) inoculation has not been tested. This research aimed to select AMF species capable of promoting growth and phosphate nutrition of this cultivar. Three clones were evaluated (M057, M194 e M199) in three Ultisols, collected in areas under sugarcane (Soil 1), Cerrado (Soil 2) and Atlantic Forest (Soil 3). The inoculation treatments were: *Acaulospora colombiana+Glomus* sp., *Acaulospora scrobiculata*, *Claroideoglomus etunicatum+Glomus* sp., *Dentiscutata heterogama*, *Gigaspora margarita* and *Rhizophagus clarus*, besides two not inoculated controls, one of them with complete fertilization. The first and third soils promoted greater vegetative plant development. The chlorophyll and phosphorus content in leaves was higher in plants of soil 3. The best response to inoculation, expressed through plant growth and nutrition, was verified in soil 1, with the best treatments being *C. etunicatum+ Glomus* sp. and *G. margarita*. The species Glomus sp., present in treatments *A. colombiana+ Glomus* sp. and *C. etunicatum+ Glomus* sp., exhibited high sporulation and promoted growth and nutrition of 'BRS Ouro Preto'. Clones M057 and M194 had the highest growth, response to inoculation and AMF sporulation, compared to clone M199. Clones M057 and M194 can, therefore, be considered mycotrophic, and promoters of AMF sporulation, while clone M199 can be classified as poorly mycotrophic.

Index terms: Arbuscular mycorrhiza, inoculant, coffee, phosphate nutrition, growth promotion.

SELEÇÃO DE FUNGOS MICORRÍZICOS ARBUSCULARES PARA *Coffea canephora* Pierre ex A. Froehner CULTIVAR CLONAL CONILON 'BRS OURO PRETO'

RESUMO: *No Brasil a cafeicultura se expande para regiões mais quentes com a cultivar clonal Conilon 'BRS Ouro Preto' da espécie C. canephora. A resposta da 'BRS Ouro Preto' à inoculação com fungos micorrízicos arbusculares (FMAs) ainda não foi testada. O presente estudo visou selecionar espécies de FMAs promotoras do crescimento e nutrição fosfatada da referida cultivar. Avaliaram-se três clones (M057, M194 e M199) em três Argissolos Vermelho-Amarelos, coletados em áreas sob: cana-de-açúcar (Solo 1), Cerrado (Solo 2) e Mata Atlântica (Solo 3). Os tratamentos de inoculação foram: Acaulospora colombiana+Glomus sp., Acaulospora scrobiculata, Claroideoglomuse tunicatum+Glomus sp., Dentiscutata heterogama, Gigaspora margarita e Rhizophagus clarus, além de duas testemunhas não inoculadas, uma delas com adubação completa. O primeiro e o terceiro solos proporcionaram maior desenvolvimento vegetativo às plantas. O teor de clorofila e fósforo nas folhas foi maior nas plantas do solo 3. A melhor resposta à inoculação, expressa através do crescimento e nutrição das plantas, foi verificada no solo 1, sendo os melhores tratamentos C. etunicatum+ Glomus sp. e G. margarita. A espécie Glomus sp., presente nos tratamentos A. colombiana + Glomus sp. e C. etunicatum+Glomus sp., apresentou alta esporulação e promoveu o crescimento e nutrição da 'BRS Ouro Preto'. Os clones M057 e M194 apresentaram maior crescimento, resposta à inoculação e esporulação de FMAs, comparados ao clone M199. Assim, os clones M057 e M194 podem ser considerados micotróficos e promotores da esporulação, enquanto o clone M199 caracteriza-se por baixa micotrofia.*

Termos para indexação: *Micorriza arbuscular, inoculante, café, nutrição fosfatada, promoção de crescimento.*

1 INTRODUCTION

Coffee is one of the main crops for Brazilian agribusiness, constantly demanding for new technologies. Reductions of dependence on imported inputs, such as, would make this crop more sustainable and profitable (RAGHURAMUKU, 2010). An alternative for achieving this goal is s the improvement of plant-microorganisms associations. Among the beneficial microorganisms for plant nutrition, coffee has been proved to associate with arbuscular mycorrhizal fungi (AMF) (SAGGIN JUNIOR; SIQUEIRA, 1996). Several studies reviewed by these authors indicate positive results of AMF inoculation in coffee plants in various cultivation stages. In nurseries, coffee generally presents low mycorrhizal colonization (SIQUEIRA et al., 1987) suggesting the use of highly fertile substrates. In lower fertility conditions, inoculation of coffee seedlings with with AMF results in good responses, expressed through plant height, dry

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matter and nutritional benefits, with higher P, Cu and Zn shoot content, combined with decreased Mn accumulation (ANDRADE; SILVEIRA; MAZZAFERA, 2010; SAGGIN JUNIOR; SIQUEIRA, 1996).

In general, coffee is considered to have high mycorrhizal dependence during seedling stage (SIQUEIRA et al., 1998). The AMF species *Gigaspora margarita*, *Rhizophagus clarus* and *Claroideoglomus etunicatum* have been reported as the most efficient in promoting seedlings growth and field survival under Cerrado soils conditions (SAGGIN JUNIOR; SIQUEIRA, 1996). Meanwhile, these results have been mainly obtained between the 1980 and 1990 decades, based on seed originated plantlets, and in *Coffea arabica* cultivars adapted and planted in Southeastern Brazil.

In present days, coffee production expands through a great diversity of regions in Brazil, even to those of lower altitudes and warmer temperatures, mainly by the use of *C. canephora* cultivars. However, there are few published studies about mycorrhizal inoculation in *C. canephora* species, and none of these makes reference to new cultivars. An example of this situation is the clonal cultivar Conilon 'BRS Ouro Preto' recently released by Embrapa and Coffee Research Consortium (*Consórcio de Pesquisa do Café*), specially developed for Western Amazonia soil and climate conditions (RAMALHO et al., 2015). Conilon 'BRS OuroPreto' represents a multiclonal cultivar, consisting of 15 vegetative multiplied clones with intermediate maturation cycles. They are adapted to the main climate features of Rondônia state, such as high temperature and air humidity, as well as moderate drought (ROCHA et al., 2015). The capacity of these clones to establish mycorrhizae has not yet been evaluated. As well, their response to inoculation with different AMF species has neither been tested. This study aimed to select AMF species which efficiently promotesgrowth and phosphate nutrition of three clones of the clonal cultivar Conilon 'BRS Ouro Preto'.

2 MATERIALS AND METHODS

The experiment was carried out under greenhouse at *Embrapa Agrobiologia*, (georeference: -22.759249° -43.680118°), in Seropédica, RJ. AMF species selection was made using three Ultisols (*Argissolos Vermelho-Amarelo*) (Table 1). Soil samples were collected from 0-20 cm layer, air dried, sieved (4 mm) and submitted to liming following recommendation for the coffee crop by the Commission on Soil Fertility of Minas Gerais State. This way, 0.98, 1.83 and 1.52 g of dolomitic lime (25% CaO, 17% MgO and 75.1% of relative power of total neutralization) were applied per kg of soils 1, 2 and 3, respectively.

The recommendation for coffee seedlings proposed by the Commission on Soil Fertility of Minas Gerais State was used as reference for fertilization of the three soils. Except for the control with complete fertilization, the others treatments received one-third (145 mg/kg of P in soil); three-quarters(187 mg/kg of K in soil) and one-quarter (75 mL/kg of compost in soil) of P, K and organic recommended fertilization using as sources potassium phosphate P.A., potassium chloride P.A. and earthworm humus.

Soil disinfection, to eliminate native AMF propagules, was made in autoclave (120 °C; 1 kgf/cm^2, 60 minutes) and repeated twice for soils 1 and 3. The soil 2, due to its probably higher Mn content, was fumigated with potassium permanganate and formaldehyde (COVACEVICH; CASTELLARI; ECHEVERRÍA, 2014). For this, the soil 2 was placed in a 166 L vacuum fumigation chamber, applied 15 mL of formaldehyde (37%) and 7.5 g of potassium permanganate and the soil was kept closed in the chamber for three days. After disinfection, independently of the method, the soils were air dried for 15 days before the experiment.

The coffee plantlets used in this study were obtained through rooting cuttings the clones M057, M194, and M199 from clonal cultivar Conilon 'BRS Ouro Preto' of *C. canephora* species. The rooting phase was conducted in 280 cm^3 cone-tainer under nursery condition at Embrapa Rondônia (georeference: -8.794007° -63.846945°).

The treatments consisted in inoculation of six AMF species (Table 2), and two not inoculated controls, one of them received complete seedling fertilization. The experimental design was factorial 3x3x8 (3 clones x 3 soils x 8 inoculation treatments) distributed in an entirely randomized design with two replicates. The experimental plot consisted of one plantlet in a cone-tainer containing 1 kg of soil. The plantlets were pruned to have just one orthotropic sprout.

AMF inoculation was applied at transplant of previously rooted cuttings to cone-tainer. For this, cuttings were taken off rooting cone-tainer (280 cm^3) and the substrate was gently removed from roots.

TABLE 1 - Soil classification, chemical characterization and georeferences of collection sites.

Classification and georeference of soil collection site	pH	Al	Ca	Mg	H+Al	K	P	C	Al Sat.
		$cmol_c/dm^3$				mg/L		%	
Soil 1-*Argissolo Vermelho-Amarelo* of arenitic formation under sugarcane (georeference: -22.138361° -50.75775°)	5.01	0.06	1.12	0.41	1.75	29	3.23	0.27	3.77
Soil 2-*Argissolo Vermelho-Amarelo* of granitic formation under Cerrado (georeference: -21.290313° -44.839346°)	5.07	0.40	0.20	0.05	2.24	19	0.62	0.12	61.54
Soil 3-*Argissolo Vermelho-Amarelo* of granitic formation under Atlantic Forest (georeference:-22.75104° -43.666254°)	4.31	0.98	0.49	0.23	4.77	23	1.92	0.75	57.65

TABLE 2 - Arbuscular mycorrhizal fungi species (AMF), identity codes, inoculum quality and quantity applied.

AMF species[1]	COFMEA[2] code	Original code	Culture code	Inoculum quality (number of spores/g)	Amount of inoculum per seedling(g)
Acaulospora colombiana (Spain & N.C. Schenck) Kaonongbua, J.B. Morton &Bever (2010) [3]	A15	CNPAB 015	1505	35	1.43
Acaulospora scrobiculata Trappe (1977)	A38	IES-33	1502	43	1.16
Claroideoglomus etunicatum (W.N. Becker &Gerd.) C. Walker & Schuessler (2010) [3]	A44	Inóculo 51	1503	08	6.25
Dentiscutata heterogama (T.H. Nicolson& Gerd.) Sieverd., F.A. Souza & Oehl (2008)	A2	CNPAB 002	1506	27	1.85
Gigaspora margarita W.N. Becker & I.R. Hall (1976)	A1	CNPAB 001	1507	18	2.78
Rhizophagus clarus (T.H. Nicolson & N. C. Schenck) C. Walker & Schuessler (2010)	A5	CNPAB 005	1561	08	6.25

[1]: Scientific names according to Redecker et al. (2013); [2]: COFMEA (Mycorrhizal Fungi Collection of *Embrapa Agrobiologia*); [3]: Although *A. colombiana* and *C etunicatum* were inoculated, just *Glomus* sp. spores were recovered from the soil of these treatments 120 days after transplant. *Glomus* sp. had high sporulation rates in coffee plants, therefore, these treatments were designated as "+*Glomus* sp". *Glomus* sp. origin is probably the roots of the seedlings colonization during cuttings rooting.

Then plants were transplanted to soils in 1 kg cone-tainer. Approximately 50 AMF spores were supplied to each plant (Table 2), with inoculum being applied over bare roots. The control treatments were not AMF inoculated. A filtered solution without AMF propagules, prepared from all used soil-inocula, was supplied to all plots (10 mL/plot) to equalize associated microbiota with inocula between treatments.

Irrigation was made with filtered water and once a week was applied per plant 50 mL of a nutrient solution containing NH_4NO_3 (80 mg/L), H_3BO_3 (927 µg/L), $CuSO_4.5H_2O$ (250 µg/L), $ZnSO_4.7H_2O$ (72 µg/L), and $Na_2MoO_4.2H_2O$ (5 µg/L). During experiment plants were sprayed with Deltamethrin (12.5 mg/L) in order to control aphids.

After 120 days from transplant, plants

were evaluated for their height, orthotropic sprout stem diameter and number of leaf pairs. Chlorophyll *a* and *b* content was measured by an electronic measuring device (CFL1030-Falker) and phosphate nutrition state by foliar discs samples, according to Aziz and Habte (1987). The disc samples were taken from the youngest totally developed leaf. P was quantified in foliar disc and P content in leaf tissue was estimated through the dry weight of disc samples. Then, the dry mass of orthotropic sprouts, cuttings, and roots was evaluated. The soil of the cone-tainers soil was sampled for quantifying the number of AMF spores after extracting them by wet sieving (GERDEMANN; NICOLSON, 1963) and centrifugation on water and sucrose. The data were analyzed for variance homogeneity and normality. The spore counting values were transformed by the square root of X+1. Data were submitted to variance analyses was and means test (Scott-Knott 5%) using SISVAR statistical program (FERREIRA, 2011).

3 RESULTS AND DISCUSSION

Table 3 exhibits means of inoculation treatments within each soil. Among the three soils, soil 1 had a significant effect of the treatment effect and soil 3 had little effect. All variables showed significant differences among inoculation treatments in soil 1. The following effects were verified in soil 1: AMF inoculated plants were higher than controls plants, with exception of those inoculated with *G. margarita*. The stem diameter was thicker in plants inoculated with *C. etunicatum*+*Glomus* sp. and *R. clarus* than others treatments. Root and sprout dry mass also were greater in plants inoculated with *C. etunicatum*+*Glomus* sp. compared to others treatments, but *G. margarita* also stimulated root dry mass. In soil 2, just stem diameter showed an effect of AMF inoculation treatments. In this case, control and *A. colombiana*+*Glomus* sp., were thicker than others treatments, suggesting the absence of real AMF inoculation effect or even a possible negative or etiolation effect. In soil 3, sprout dry mass was the only variable that showed a difference, with the treatments *A. scrobiculata*, *C. etunicatum*+ *Glomus* sp. and *D. heterogama* having lower values than others, including controls, suggesting random effect.

The inoculation treatments did not reveal any effect on the number of, nor on n the dry ass of the cuttings therefore these data were not presented. The absence of effect on cuttings dry mass indicates cuttings do not have a significant development after plantlet transplant and initial conditions of plantlets were uniform. Chlorophyll *a* content was even less influenced by treatments than chlorophyll *b*, thus these data were also not presented. The highest chlorophyll *b* contents were verified on soil 1 in plants with *A. colombiana*+*Glomus* sp. and *C. etunicatum*+*Glomus* sp., as well as in those of controls with complete fertilization. In soil 2, there was no effect of inoculation on chlorophyll *b* contents. In soil 3, the highest values occurred on *A. scrobiculata* and control with complete fertilization.

The number of AMF spores in the soils after the experiment was not different between treatments in soil 2. However, in the other two soils, differences were well marked. In soil 1, *Glomus* sp. present in *A. colombiana*+ *Glomus* sp. and *C. etunicatum*+ *Glomus* sp. had higher sporulation compared the other other AMF species tested. Other treatments also showed sporulation but did not differ from controls without sporulation due to high data variability (variation coefficient of 97.3%). In soil 3, sporulation patterns between different AMF species were better defined. As in soil 1, in soil 3 *Glomus* sp has the highest sporulation, being superior to other treatments. Other species, as *A. scrobiculata* and *R. clarus* also had a high number of spores, being superior from controls. On the other side, sporulation of *G. margarita* and *D. heterogama* were not different from controls.

Table 4 displays means of inoculation treatments within each clone. Effect of inoculation treatments on growth variables and on chlorophyll *b* content was little within each clone. In clone M057, root dry mass was heavier in treatment with *G. margarita* and chlorophyll *b* content was higher in treatments with *A. colombiana*+ *Glomus* sp., *A. scrobiculata* and *C. etunicatum*+ *Glomus* sp., as well as in control with complete fertilization. In Clone M194, inoculation effect was seen only for stem diameter with *A. colombiana*+ *Glomus* sp., *C. etunicatum*+ *Glomus* sp., *R. clarus,* and control treatments exhibiting greater greater diameter than other. In clone M199, inoculation with *A. colombiana*+ *Glomus* sp., *R. clarus*, and control promoted thicker stem diameter the other treatments. At the same time, in this clone, *A. colombiana*+*Glomus* sp. and control with complete fertilization stimulated higher chlorophyll *b* contents.

TABLE 3 - Plant height, sprout stem diameter, root and sprout dry mass, foliar content of chlorophyll *b* and Glomeromycota number of spores in seedlings of *C. canephora* cultivar Conilon 'BRS Ouro Preto' under different arbuscular mycorrhizal fungi (AMF) inoculation treatments in three soils.

AMF inoculation treatment	Plant height (cm)	Sprout stem diameter (mm)	Roots dry mass (g)	Sprout dry mass (g)	Chlorophyll *b* content (ICF)	Number of spores in 50 mL of soil*
Soil 1-*ArgissoloVermelho-Amarelo* of arenitic formation under sugarcane						
A. col.+*Glomus* sp	13.67**a**	4.08**b**	1.29**b**	3.41**b**	8.17**a**	18.89**a**
A. scrobiculata	14.28**a**	4.30**b**	1.20**b**	3.27**b**	6.25**b**	7.38**b**
C. etu.+*Glomus* sp	17.80**a**	4.86**a**	2.26**a**	5.00**a**	7.18**a**	22.71**a**
D. heterogama	14.42**a**	4.11**b**	1.33**b**	3.75**b**	5.93**b**	3.24**b**
G. margarita	10.60**b**	4.02**b**	1.69**a**	2.85**b**	6.08**b**	1.72**b**
R. clarus	14.83**a**	4.71**a**	1.34**b**	3.78**b**	5.23**b**	9.88**b**
N.I. control+Fert.	12.00**b**	4.00**b**	0.81**b**	3.46**b**	7.47**a**	1.00**b**
N.I. control	11.00**b**	4.41**b**	1.03**b**	2.82**b**	5.20**b**	1.00**b**
Soil 2-*Argissolo Vermelho-Amarelo* of granitic formation under Cerrado						
A. col.+*Glomus* sp	13.17a	4.93**a**	0.93a	3.15a	5.73a	4.27a
A. scrobiculata	11.47a	4.14**b**	0.94a	2.73a	5.45a	5.25a
C. etu.+*Glomus* sp	10.68a	4.17**b**	0.70a	2.17a	5.07a	8.32a
D. heterogama	12.43a	4.29**b**	0.97a	2.88a	6.15a	1.58a
G. margarita	12.90a	4.27**b**	1.26a	3.11a	5.58a	1.53a
R. clarus	10.50a	4.49**b**	0.91a	2.40a	5.50a	4.85a
N.I. control+Fert.	9.58a	4.00**b**	0.71a	1.78a	4.72a	1.00a
N.I. control	14.00a	5.17**a**	1.78a	3.36a	5.20a	1.00a
Soil 3-*Argissolo Vermelho-Amarelo* of granitic formation under Atlantic Forest						
A. col.+*Glomus* sp	15.55a	4.92a	1.95a	5.14**a**	7.10**b**	15.18**a**
A. scrobiculata	15.80a	4.47a	1.30a	3.84**b**	7.86**a**	11.45**b**
C. etu.+*Glomus* sp	16.42a	4.60a	1.99a	4.15**b**	6.78**b**	18.95**a**
D. heterogama	14.75a	4.32a	1.77a	3.90**b**	6.15**b**	4.32**c**
G. margarita	15.38a	4.53a	2.01a	4.82**a**	6.93**b**	3.01**c**
R. clarus	16.15a	4.85a	1.75a	4.97**a**	5.93**b**	9.07**b**
N.I. control+Fert.	15.50a	4.56a	1.52a	5.26**a**	8.98**a**	1.00**c**
N.I. control	15.45a	4.68a	1.60a	4.69**a**	5.98**b**	1.00**c**

Abbreviations used: *A.= Acaulospora; col= colombiana; C. etu.= Claroideoglomus etunicatum; D.= Dentiscutata; G.= Gigaspora; R.= Rhizophagus;* N.I. control= Not inoculated control; Fert.= Complete fertilization recommended for production of coffee seedlings; ICF= Falker Chlorophyll Index. *: square root of X+1 (transformed data). Letters in columns, within each soil, compare means by Scott-Knott 5% test.

TABLE 4 - Plant height, sprout stem diameter, root and sprout dry mass, foliar content of chlorophyll *b* and Glomeromycota number of spores in seedlings of three clones of *C. canephora* cultivar Conilon 'BRS Ouro Preto' under different arbuscular mycorrhizal fungi (AMF) treatments.

AMF inoculation treatment	Plant height (cm)	Sprout stem diameter (mm)	Roots dry mass (g)	Sprout dry mass (g)	Chlorophyll *b* content (ICF)	Number of spores in 50 mL of soil*
Clone M057						
A. col.+*Glomus* sp	11.65a	4.52a	1.35**b**	4.11a	8.38**a**	19.52**a**
A. scrobiculata	10.46a	4.49a	0.89**b**	3.39a	6.82**a**	6.01**b**
C. etu.+*Glomus* sp	10.10a	4.54a	1.54**b**	3.41a	6.78**a**	15.39**a**
D. heterogama	8.80a	4.33a	1.04**b**	3.42a	5.68**b**	3.06**b**
G. margarita	10.30a	4.45a	2.33**a**	4.31a	5.98**b**	2.31**b**
R. clarus	10.70a	4.79a	1.51**b**	4.27a	5.70**b**	12.26**a**
N.I. control+Fert.	9.08a	4.35a	1.02**b**	3.79a	7.28**a**	1.00**b**
N.I. control	9.88a	4.54a	1.36**b**	4.39a	5.88**b**	1.00**b**
Clone M194						
A. col.+*Glomus* sp	18.34a	4.47**a**	1.65a	4.74a	5.54a	10.68**b**
A. scrobiculata	15.98a	3.90**b**	1.01a	3.20a	6.87a	12.78**b**
C. etu.+*Glomus* sp	18.67a	4.53**a**	1.72a	4.36a	6.77a	24.92**a**
D. heterogama	17.83a	4.17**b**	1.56a	3.88a	7.60a	3.94**c**
G. margarita	16.92a	4.05**b**	1.32a	3.71a	6.78a	2.25**c**
R. clarus	18.87a	4.41**a**	1.20a	4.12a	6.32a	10.12**b**
N.I. control+Fert.	14.08a	3.82**b**	0.91a	3.26a	7.15a	1.00**c**
N.I. control	14.88a	4.39**a**	1.26a	3.59a	5.80a	1.00**c**
Clone M199						
A. col.+*Glomus* sp	11.83a	5.39**a**	1.56a	3.45a	7.70**a**	9.70a
A. scrobiculata	14.22a	4.51**b**	1.48a	3.17a	5.70**b**	4.38a
C. etu.+*Glomus* sp	14.26a	4.43**b**	1.43a	2.95a	5.06**b**	5.59a
D. heterogama	14.97a	4.22**b**	1.47a	3.23a	4.95**b**	2.15a
G. margarita	11.23a	4.34**b**	1.48a	2.96a	5.90**b**	1.82a
R. clarus	11.92a	4.87**a**	1.29a	2.76a	4.65**b**	1.41a
N.I. control+Fert.	13.92a	4.39**b**	1.10a	3.45a	6.73**a**	1.00a
N.I. control	14.74a	4.80**a**	1.42a	3.30a	5.12**b**	1.00a

Abreviations used: *A.= Acaulospora; col= colombiana; C.etu.= Claroideoglomus etunicatum; D.= Dentiscutata; G.= Gigaspora; R.= Rhizophagus;* N.I. control= Not inoculated control; Fert.= Complete fertilization recommended for the prduction of coffee seddlings; ICF= Falker Chlorophyll Index. *: square root of X+1 (transformed data). Letters in columns, within each clone, compare means by Scott-Knott 5% test.

About the number of spores, clone M199 did not promote differences among inoculation treatments, with sporulation being low in all of them, not getting to be different from controls without sporulation. In clone M057, inoculation with *A. colombiana+Glomus* sp., C. *etunicatum+Glomus* sp., and *R. clarus* resulted in larger sporulation than others treatments. In clone M194, the higher sporulation occurred on *C. etunicatum+Glomus* sp., followed by *A. colombiana+Glomus* sp., *A. scrobiculata,* and *R. clarus*. Sporulation of *G. margarita* and *D. heterogama* did not differ from controls.

The soil and clone main effects are presented in Table 5. It was verified that soil 3 propitiated the best development of plants, as well as the highest content of chlorophyll b. Soil 1 showed a better plant development compared to soil 2, and similar or slightly inferior compared to soil 3. Soil 2 promoted the lowest plant development and chlorophyll *b* content. AMF sporulation was also higher in soils 1 and 3, being in both cases higher in comparison to soil 2.

The clone main effect was variable about to plant growth responses. Clone M194 had higher plants, but thinner sprout stem diameter. Clone M057 had lower height, but a greater number of leaves (data not presented). Clone M057 had a higher dry mass of cuttings compared to the others clones (data not presented). The clones did not differ in relation to root mass, but clones M057 and M194 had more sprout dry mass and chlorophyll *b* content compared to clone M199. Clones M057 and M194 also promoted greater AMF sporulation than clone M199.

The phosphorus nutrition status of plants is presented in Figure 1. AMF inoculation did not influence tissues P content in the three soils. However, P quantity in leaf discs showed differences among inoculation treatments in soils 2 and 3. In soil 2, inoculated treatments, except *R. clarus*, promoted higher P quantities in leaf discs compared to controls. In soil 3, inoculation with *A. colombiana+Glomus* sp., *C. etunicatum+Glomus* sp. and *G. margarita* stimulated higher P quantities in leaf discs.

The analyses of inoculation treatments within each clone (Figure 2) indicated a positive effect of most AMF species in tissue P content of clone M057. Except for *D. heterogama,* all others inoculated AMF species promote higher P content than controls. Regarding P quantity in leaf disc of clone M057, the inoculation with *A. colombiana + Glomus* sp. provided the highest values, followed by *C. etunicatum+Glomus* sp. and *G. margarita* treatments. In clone M194, all treatments AMF inoculated promoted higher P quantities in leaf discs compared to controls, including the control with complete fertilization. On the other side, clone M199, did not have any positive effect on P nutrition status due to mycorrhizal inoculation.

The soil and clone main effects about phosphorus nutrition are in Figure 3. Soil 3 propitiated higher P accumulations in leaf discs, as well as higher tissue P content, compared to the other two soils.

TABLE 5 - Plant height, sprout stem diameter, root and sprout dry mass, foliar content of chlorophyll *b* and Glomeromycota number of spores in seedlings of three clones of *C. canephora* cultivar Conilon 'BRS Ouro Preto' grown in three different soils (Main effects of clone and soil).

Soil/Clone treatment	Plant height (cm)	Sprout stem diameter (mm)	Roots dry mass (g)	Sprout dry mass(g)	Chlorophyll *b* content (ICF)	Number of spores in 50 mL of soil*
Soil main effect						
Soil1	13.37**b**	4.30**b**	1.33**b**	3.48**b**	6.28**b**	6.81**a**
Soil2	11.44**c**	4.30**b**	0.93**c**	2.57**c**	5.43**c**	3.78**b**
Soil3	15.62**a**	4.62**a**	1.74**a**	4.61**a**	6.95**a**	7.92**a**
Clone main effect						
Clone M057	10.05**c**	4.50**a**	1.37a	3.86**a**	6.51**a**	7.28**a**
Clone M194	17.01**a**	4.20**b**	1.33a	3.85**a**	6.66**a**	8.61**a**
Clone M199	13.44**b**	4.56**a**	1.39a	3.14**b**	5.62**b**	2.95**b**

Soil 1 - *Argissolo Vermelho Amarelo* (AVA) of arenitic formation under sugarcane; **Soil 2**-AVA of granitic formation under Cerrado; **Soil 3**-AVA of granitic formation under Atlantic Forest. Letters in columns within soil or clone effect, compare means by Scott-Knott 5% test.

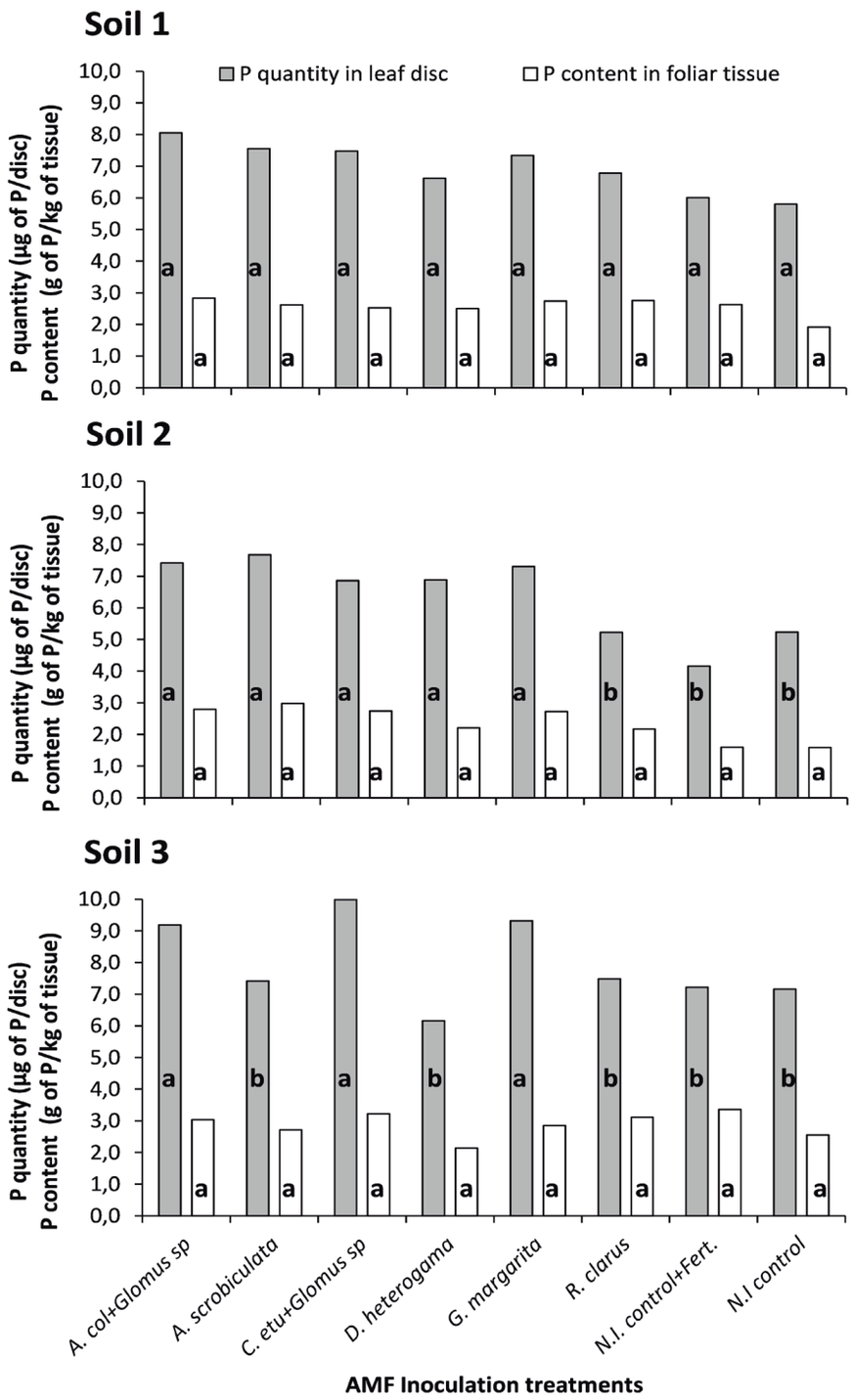

FIGURE 1 - P quantity in leaf disc and P content in foliar tissue in seedlings of *C. canephora* cultivar Conilon 'BRS Ouro Preto' under different arbuscular mycorrhizal fungi (AMF) inoculation treatments in three soils. Used abbreviations: *A.= Acaulospora; col= colombiana; C. etu.= Claroideoglomuse tunicatum; D.= Dentiscutata; G.= Gigaspora; R.= Rhizophagus;* N.I. control= Not inoculated control; Fert.= Complete fertilization recommended for coffee seedlings production; Letters within each bar color, and within each soil, compare means by Scott-Knott 5% test.

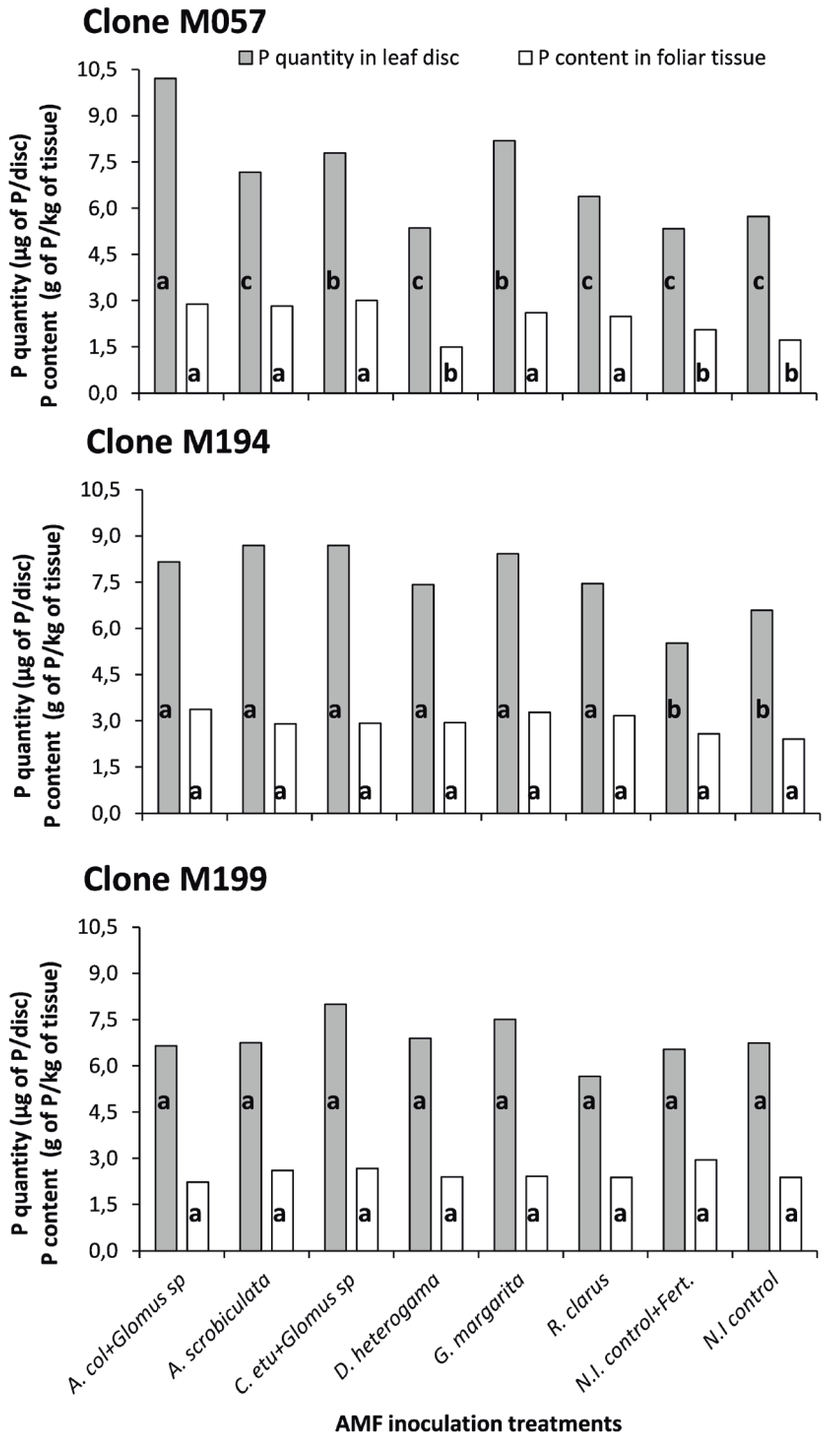

FIGURE 2 - P quantity in leaf disc and P content in foliar tissue in seedlings of three clones *C.canephora* cultivar Conilon 'BRS Ouro Preto' under different arbuscular mycorrhizal fungi (AMF) inoculation treatments. Used abbreviations: *A.= Acaulospora; col= colombiana; C.etu.= Claroideoglomus etunicatum; D.= Dentiscutata; G.= Gigaspora; R.= Rhizophagus*; N.I. control= Not inoculated control; Fert.= Complete fertilization recommended for coffee seedlings production; Letters within each bar color, and within each clone, compare means by Scott-Knott 5% test.

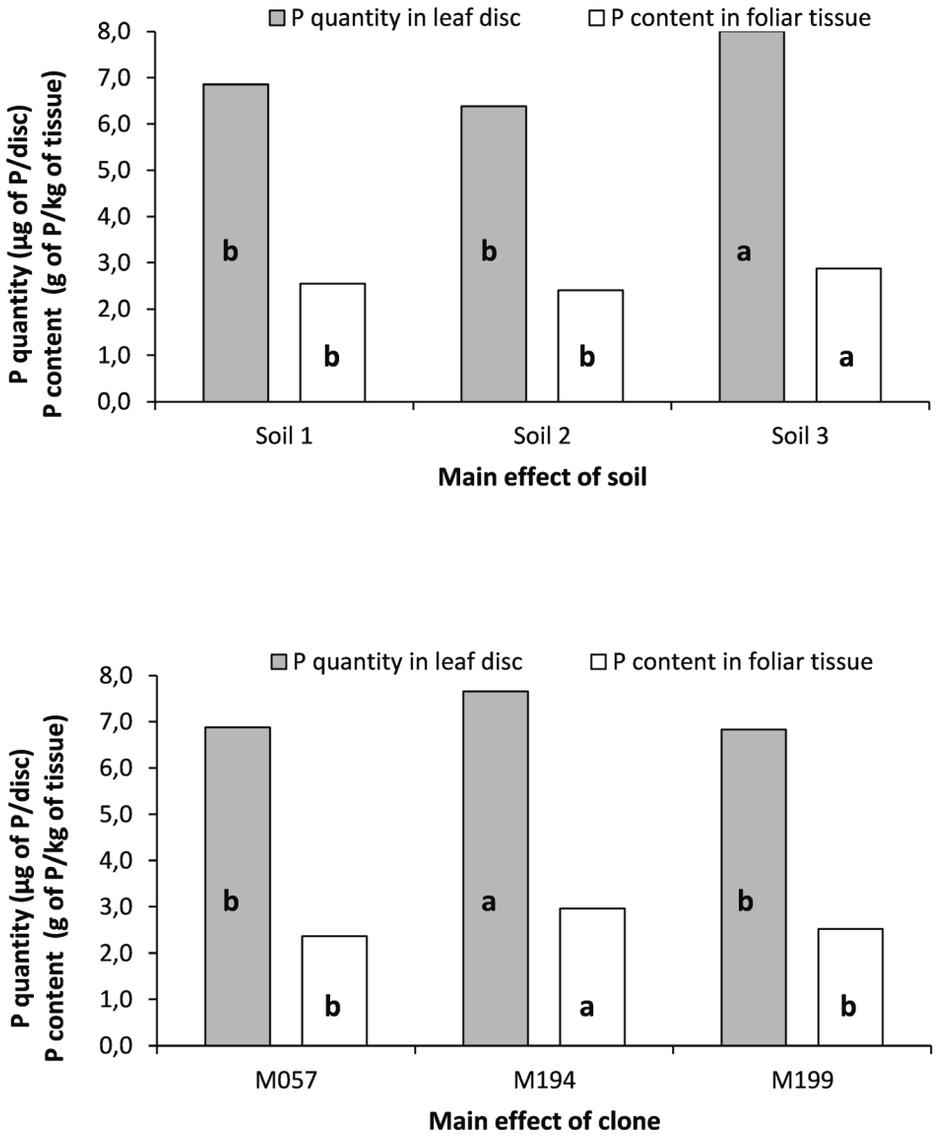

FIGURE 3 - P quantity in leaf disc and P content in foliar tissue in seedlings of three clones of *C. canephora* cultivar Conilon 'BRS Ouro Preto'cultivated in three soils. Letters within each bar color compare means by Scott-Knott 5% test.

This is in correspondence with the higher P availability in this soil, which in general presented a better fertility (Table 1). The clone M194 showed the best P absorption, expressed through higher P quantities in leaf discs and higher tissue P content, in comparison to the other two clones. This suggests thats clone M194 is more efficient on P absorption and/or P accumulation in leaves than clones M057 and M199.

Among the three studied soils, soil 3 stimulated the highest growth of coffee Conilon 'BRS Ouro Preto'. The plants in this soil had higher leaf chlorophyll and P contents. Although this soil had initially presented the highest acidity and aluminum saturation (Table 1), these problems were solved through liming. This soil, collected under Atlantic Forest vegetation, had higher carbon content compared to the others two soils, which certainly resulted in higher fertility levels and therefore explain the obtained results.

The development of the coffee seedlings in soi 1 was basically the same or slightly inferior compared to plants grown in soil 3. In general plants in soil 1 grew better that those in soil 2,

being soil 2 the one with the poorest fertility, resulting in the smallest plants, with a lower number of leaves, chlorophyll content and dry mass production. Soil 2 was originally from Cerrado and had very low levels of Ca, Mg, P and C combined with high aluminum saturation (Table 1). Compared to the other two soils evaluated, soil 2 became compact and hard within the cone-tainers. These chemical and physical differences between soils might justify the poorest development of plants associated with soil 2. However, in addition, different from the other two soils, soil 2 was fumigated with the gas resulting from the mixture of potassium permanganate and formaldehyde (COVACEVICH; CASTELLARI; ECHEVERRÍA, 2014), which may also have affected is fertility and decreased the establishment of AMF, since soil 2 showed low sporulation after conducting experiments.

The highest response of coffee plants to AMF inoculation, expressed through growth and P nutrition, were observed in soil 1. Soils 2 and 3 promoted low levels of inoculation response, as they were soils with respective highest and lowest fertilities. The larger effect of AMF inoculation in coffee generally takes place in intermediate-fertility conditions (SAGGIN JUNIOR; SIQUEIRA, 1996), as verified in soil 1. In addition, P and N fertilization affect AMF communities in coffee roots (DE BEENHOUWER et al., 2015).

The plants of clone M194 showed higher plants, but thinner sprout stem diameter, suggesting a probable etiolated growth. On the other side, plants of clone M057 had a lower height, but a greater number of leaf pairs, indicating clone M057 had more branches. Clone M057 had a higher dry mass of cuttings than the other clones, which suggests mother plants more vigorous. Despite these differences in height, stem diameter, number of leaf pairs, and cuttings dry mass, the quantification of plants total dry mass showed clones M057 and M194 grew more than clone M199. Clones M057 and M194 had a better response to AMF inoculation, combined with a higher fungal sporulation. This suggests clones M057 and M194, mainly the first one, are more mycotrophic compared to clone M199. This last clone (M199) had a low AMF sporulation and under short fertilization conditions (1/3 of P, 3/4 of K and 1/4 of organic fertilization) showed seedlings with low growth and low chlorophyll content.

The selection of better performance mother plants to compound clonal cultivars, as cultivar Conilon 'BRS Ouro Preto', should be done based on an additional criterion, which rarely is taken into account by plant breeders, which is the capacity of these plants to associate with microorganisms capable of improving their nutrition. When selection is performed under high fertilization conditions, exists the risk that selected plants are productive only in highly fertile situations and that selected plants do not share photosynthetic products (not have energy expenditure) with root symbionts. This might have been the case of clone M199, which had a low association with AMF. Selection of highly productive plants that only respond to high fertilizer application or to very fertile soils puts plant breeding against the solution for the main Brazilian agribusiness problem, which is a high dependency on imported chemical fertilizers. For agribusiness to become less dependent on fertilizers, it is necessary to select plants capable of using alternative nutritional strategies, as association with AMF. Reducing use of chemical fertilizers in coffee plantations would increase its sustainability (FERNANDES et al., 2016) and AMF mycelium may result in a greater nutrient acquisition from organic sources in soils with low nutrient availability (POSADA; SIEVERDING, 2014).

In general, it was verified coffee grew better in soil 1 under inoculation treatments of *C. etunicatum+Glomus* sp. and *G. margarita*. The higher sporulation of *Glomus* sp. in treatments *A. colombiana+Glomus* sp. and *C. etunicatum+Glomus* sp., in soils 1 and 3, and in clones M057 and M194 suggests this AMF species is well adapted to 'BRS Ouro Preto'. The fungi *A. scrobiculata* and *Rhizophagus clarus* also showed good sporulation, with the latter species also promoting shoot growth of plants.

4 CONCLUSIONS

Soil 3 (from Atlantic Forest) was the most fertile, promoted greater coffee plants, with higher chlorophyll content and better phosphate nutrition. However, this soil decreases the response of plants to AMF inoculation. Soil 1 (from sugarcane plantation) of intermediate fertility, between other two soils, promoted good development and AMF responsive coffee plants. Clones M057 and M194 have higher growth compared to M199 in studied soils and on fertilization conditions lower than recommended for coffee seedlings. Clones M057 and M194, mainly the former, are mycotrophic and promote sporulation of inoculated fungi, while clone M199 has low

mycotrophy. The fungus *Glomus* sp. present in treatments *A. colombiana+Glomus* sp. and *C. etunicatum+Glomus* sp., in general, had high sporulation and promotes growth and nutrition of cultivar Conilon 'BRS Ouro Preto'. The fungi *A. scrobiculata*, *R. clarus* and *G. margarita* also stimulated the growth of coffee seedlings.

5 ACKNOWLEDGMENTS

This study was conducted within the Activity "AMF selection for *C. canephora* seedlings" associated with the project "Arbuscular Mycorrhizal Fungi and microbial interactions in coffee plantations in the Amazon" do PNP&D/ Café, with Funcafé funds.

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