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INFLUENCE OF ARBUSCULAR MYCORRHIZAL FUNGI ON BIOLOGICAL CONTROL OF COFFEE LEAF RUST (*Hemileia vastatrix* BERK. & BROOME)

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ABSTRACT

Inoculating plants with arbuscular mycorrhizal fungi (AMF) protects host plants against biotic stressors such as diseases. Therefore, the objective of this study was to evaluate the influence of arbuscular mycorrhizal fungi in the biological control of coffee leaf rust (CLR) infections. The research involved two varieties of coffee (Caturra and Pache) and three inocula of AMF: Moyobamba (*Acaulospora mellea*, *Acaulospora* sp.1, *Glomus geosporum*, *Glomus* sp.1, and *Glomus* sp.2), El Dorado (*Acaulospora rugosa*, *Acaulospora spinosissima*, *Acaulospora lacunosa*, *Glomus sinuosum* and *Ambispora appendicula*) and Huallaga (*Acaulospora mellea*, *Acaulospora* sp.1, *Acaulospora* sp.2, *Glomus macrocarpum* and *Glomus* sp.2), in addition to a control treatment without application of AMF (non-AMF). Inoculation of vegetatively propagated coffee plants with AMF was observed to induce tolerance to CLR. The incidence of CLR in non-AMF coffee plants was 43.7 %, while in the coffee plants subjected to the inocula Moyobamba, El Dorado and Huallaga, the incidences were 22.1, 22.7 and 13.2 %, respectively. Likewise, the severity in non-AMF coffee plants was 34.8 %, while in the coffee plants subjected to the three kinds of inocula, the severities were 21.1, 20.4 and 12.0 %, respectively. Thus, mycorrhizal inoculation of coffee plants at the nursery stage reduces the negative effect of CLR infection after the plants are taken to field conditions, representing a sustainable option for their biological control.
Additional keywords: Crop protection, mycorrhizal inoculation, sustainable agriculture, symbiosis

RESUMEN

Influencia de los hongos micorrícicos arbusculares en el control biológico de la roya amarilla del café (*Hemileia vastatrix* Berk. & Broome)

La inoculación de plantas con hongos micorrízicos arbusculares (HMA) induce protección contra factores estresantes bióticos como las enfermedades. Por lo tanto, este estudio tuvo como objetivo evaluar la influencia de los hongos micorrízicos arbusculares en el control biológico de la roya amarilla del café. Para ello, se utilizaron dos variedades de café (Caturra y Pache) y tres inóculos de HMA: Moyobamba (*Acaulospora mellea*, *Acaulospora* sp.1, *Glomus geosporum*, *Glomus* sp.1 y *Glomus* sp.2). El Dorado (*Acaulospora rugosa*, *Acaulospora spinosissima*, *Acaulospora lacunosa*, *Glomus* sp.1 y *Ambispora appendicula*) y Huallaga (*Acaulospora mellea*, *Acaulospora* sp.1, *Acaulospora* sp.2, *Glomus macrocarpum* y *Glomus* sp.2), además de un control sin aplicación de HMA. Las plantas de café propagadas vegetativamente e inoculadas con HMA mostraron tolerancia a la roya amarilla. La incidencia de roya amarilla en plantas que no fueron inoculadas con HMA fue de 43,7 %, mientras que en las plantas inoculadas con Moyobamba, El Dorado y Huallaga, las incidencias fueron 22,1; 22,7 y 13,2 %, respectivamente. Asimismo, la severidad en plantas que no fueron inoculadas fue de 34,8 %, mientras que en las plantas de café sometidas a los inóculos Moyobamba, El Dorado y Huallaga, las severidades encontradas fueron 21,1; 20,4 y 12,0 %, respectivamente. Se concluye que la inoculación micorrícica de plantas de café en etapa de vivero reduce los efectos negativos de la infección por roya amarilla luego de llevadas a condiciones de campo, y representa una opción sustentable para su control biológico.
Palabras clave adicionales: Agricultura sostenible, inoculación micorrícica, protección de cultivos, simbiosis

INTRODUCTION

Coffee (*Coffea arabica* L.) is one of the most valuable agricultural export products of tropical regions (Herrera et al., 2019), and its production and commercialization requires much work from the different actors involved in its value chain.

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Being a perennial crop, coffee presents certain susceptibility to climatic changes, with negative effects that mostly range from low production and quality, to marked exposure to diseases and pests (Pham et al., 2019). In 2008, an outbreak of coffee leaf rust (CLR) appeared throughout Latin America (McCook and Vandermeer, 2015), seriously affecting the region, and generating productivity losses (Juárez et al., 2018). This revealed that the main risks farmers have for agricultural production include outbreaks of pests and diseases (Morton, 2007). CLR is caused by *Hemileia vastatrix*, a biotrophic fungus that affects the leaves, which is why it is considered the most serious disease in coffee production worldwide (Avelino et al., 2015), since it can cause yield losses from 30 to 100 % (Piato et al., 2020).

The main strategies for the management of the CLR have focused on the use of agro-pesticides. Therefore, research is needed to improve the sustainable and efficient management of this disease in coffee plantations. A study carried out in Costa Rica reported that rains are the main driver of the dispersion of *H. vastatrix* uredospores in coffee plantations under shade, while in full sun wind inhibited the accumulation of water on the leaves reversing this effect (Boudrot et al., 2016). Thus, the shade percentage, rain, and wind conditions are other factors involved in the presence of the foliar disease in coffee plants.

Arbuscular mycorrhizal fungi (AMF) are symbiotic rhizospheric microorganisms that exchange benefits with plants. AMF enhance the absorption of nutrients by developing a mycelial network in the rhizosphere, stimulating the vegetative growth, and inducing tolerance to phytopathogens, as well as the adaptation of crops to abiotic stress conditions, all this in exchange for photosynthetic products (Aguilera et al., 1999; Marro et al., 2014). Plant diseases can be controlled by using indigenous microorganisms or by applying antagonists. The symbiotic interaction between AMF and host plants has a positive effect in reducing the severity of plant diseases in various crops (Aljawasim et al., 2020; Weng et al., 2022). A study published by Vallejos et al. (2021) showed that the inoculation of coffee plants with AMF prior to field establishment protects host plants against root-knot nematode infection. In this sense, the use of these soil microorganisms is a potential tool for the biological control of plant pathogens.

Based on the previous lines, the objective of this study was to evaluate the influence of arbuscular mycorrhizal fungi in reducing the damage caused by leaf rust in vegetatively propagated coffee plants installed under field conditions.

MATERIAL AND METHODS

Study area. The research was carried out in experimental plots located in the Jepelacio district, Moyobamba province and San Martín region, Peru, at an altitude of 1050 m, with geographical coordinates 6º 10' S, 76º 53' W. The environmental conditions of the research plot were registered from October 2019 to July 2020 (Table 1). The soil properties of the experimental plots are indicated in Table 2.

Table 1. Climatic data during the study period

Month	Temperature max/min (C°)	Relative humidity (%)	Precipitation (mm)
Oct	29.4/15.2	91.4	227.5
Nov	30.1/15.6	90.8	219.5
Dec	30.6/16.2	90.4	225.9
Jan	30.4/16.0	90.1	173.3
Feb	30.4/17.0	90.5	197.8
Mar	30.8/17.2	89.7	155.2
Apr	28.6/14.2	88.8	170.2
May	30.8/16.8	88.5	150.3
Jun	27.2/16.8	89.1	137.0
Jul	28.0/17.2	89.6	131.0

Experimental design. In this study, a factorial arrangement with two factors and eight treatments was used. The factors under study were factor A - Coffee variety (Caturra and Pache), and factor B - AMF inocula (Non-AMF, Moyobamba inoculum, El Dorado inoculum, and Huallaga inoculum). Three replicates for each treatment were installed. The treatments were randomly distributed during the field installation. Considering that the research was carried out under field conditions, two rows of coffee plants were placed between each repetition to avoid cross-contamination into the experiment. The control treatment corresponded to vegetatively propagated coffee plants that were not inoculated with AMF, and which were only influenced by the native soil microorganisms of the study area.

Table 2. Soil properties of the experimental plots

Source	Electrical conductivity (dS/m)	pH	Organic matter (%)	N (%)	P (ppm)	K (ppm)
Caturra - Non-AMF	0.62	6.81	3.81	0.17	46.89	854
Pache - Non-AMF	0.45	6.60	5.71	0.26	16.86	396
Caturra – Moyobamba inoculum	0.27	6.42	4.86	0.22	11.64	513
Pache -Moyobamba inoculum	0.28	6.43	5.40	0.24	13.72	272
Caturra - El Dorado inoculum	0.36	7.16	5.39	0.24	24.71	320
Pache - El Dorado inoculum	0.17	6.63	3.32	0.15	7.82	381
Caturra - Huallaga inoculum	0.12	6.88	4.38	0.20	13.55	409
Pache - Huallaga inoculum	0.34	7.49	4.49	0.20	13.37	364

Vegetative propagation of coffee plants. Coffee plants vegetatively propagated by rooting cuttings were used. For this purpose, cuttings 8 cm long, with 50 % of leaf area, in Jiffy pellets receiving 2000 $mg \cdot L^{-1}$ of indolebutyric acidy were used (Vallejos et al., 2020).

The rooting induction period of coffee cuttings in microtunnels was two months, then the rooted cuttings were transferred to greenhouse bags using a substrate which contained sterile river sand and agricultural soil in a 1:2 proportion.

Inoculation of coffee plants with AMF. The AMF inocula used were obtained from the Phytopathology Laboratory of the Instituto de Investigaciones de la Amazonía Peruana. Table 3 indicates the species that contain each AMF inoculum.

Table 3. Identified species of AMF in each inoculum

AMF inoculum	Identified species of AMF
Moyobamba inoculum	*Acaulospora mellea, Acaulospora* sp1., *Glomus geosporum, Glomus* sp.1 y *Glomus* sp.2
El Dorado inoculum	*Acaulospora rugosa, Acaulospora spinosissima, Acaulospora lacunosa, Glomus sinuosum y Ambispora appendicula*
Huallaga inoculum	*Acaulospora mellea, Acaulospora* sp.1, *Acaulospora* sp.2, *Glomus macrocarpum y Glomus* sp.2

The vegetatively propagated coffee plants were inoculated with the AMF inocula while they were transferred to the greenhouse bags. For this purpose, about 2000 spores of AMF were sprinkled on the substrate (Vallejos et al., 2019). The coffee plants inoculated with the AMF inocula were placed in a greenhouse with 80 % shade for 15 days and then they were transferred

to another greenhouse with 50 % shade for 15 more days. Subsequently, the mycorrhized coffee plants were exposed to sunlight for five months, then they were infected with *H. vastatrix*, and finally installed in field conditions.

Infection of coffee plants with *H. vastatrix*. To obtain the inocula of *H. vastatrix*, leaves infected with CLR were collected from coffee plantations located in the nearby province of Lamas. The infected leaves were selected for the presence of yellowish-orange chlorotic spots, and then they were collected, stored, and transported in dry plastic containers to the Phytopathology laboratory of the Instituto de Investigaciones de la Amazonía Peruana - San Martín. The uredospores obtained from infected plants are the main inoculum of *H. vastatrix*. Following the recommendations of Costa et al. (2007), the coffee leaves with signs of CLR infection were placed on bond paper. Then, with the help of a soft bristle brush #6, the uredospores were removed from the fungal pustules located on the adaxial side of the leaves. Uredospores of *H. vastatrix* were transferred to sterile distilled water at a concentration of 4 mg·mL^{-1}. With the help of a micropipette, the inoculant solution of CLR was extracted and placed in the Neubauer chamber where the uredospores were counted using the stereomicroscope, and the concentration per milliliter was determined. The CLR inoculants were formulated at a concentration of 1x10^5 uredospores·mL^{-1}.

The solutions with CLR inoculant were sprinkled on the underside of the coffee leaves using a manual sprinkler at a rate of 2 mL per plant. The coffee plants were inoculated with *H. vastatrix* using an atomizer to obtain a uniform spray on the plants, four months after inoculation with the AMF inocula and after verifying that the roots colonization with AMF of coffee plants was greater than 20 %.

Field installation of coffee plants and evaluation of variables. Five months after the inoculation of AMF, and one month after the intentional infection with uredospores of *H. vastatrix*, the coffee plants were installed spaced at 1.5 m x 1.5 m in the experimental plots under field conditions, and one kilogram of compost was applied for each plant.

The evaluations were carried out 10 months after the installation of the coffee plants in the experimental plots, when plant height and number of branches were measured per treatment.

On the other hand, 10 g of soil samples with secondary roots were collected from each one of the coffee plants, at a distance of 30 cm from the main stem and 15 cm soil deep. The collected soil samples were placed in plastic bags with hermetic seal and transferred to the laboratory for drying at room temperature. The secondary roots were obtained following the methodology adapted by Vallejos et al. (2019), and the method reported by Phillips and Hayman (1970) was used for the staining of secondary roots. The stained roots were preserved in white vinegar. For the evaluation of mycorrhizal colonization was followed the method reported by Brundrett et al. (1996). For this purpose, 20 root segments of 1.5 cm of each coffee plant were isolated and placed in microscope slides to be observed. Then, each segment of the root was divided into three observation areas under the microscope (upper, middle, and lower). Each area was carefully observed, and the presence or absence of mycorrhizal structures was identified: vesicles, arbuscules and hyphae. The calculation of the mycorrhizal colonization of AMF in the roots was carried out through the following formula:

Mycorrhizal colonization (%) = (n / N) x 100

where N = Total number of segments evaluated; and n = Total number of areas with presence of mycorrhizal structures.

The staining of the extra-radical mycelium was done using one gram of soil sample and the semisolid gel technique. To quantify the length of the extra-radical mycelium, the quadrant intersection method was used. For this, 10 mL of the solution obtained through the semisolid gel technique were taken and placed in a Petri dish. Millimeter paper sheet was placed at the outer base of the Petri dish. The samples placed in the Petri dishes were observed in a stereoscope at 4.5 X magnification fir quantifying the hyphal-line intersections. The numerical quantity obtained was transformed into extra-radical mycelium length per unit of soil weight using the formula proposed by Newman (1966):

$$R = \pi AN/2H$$

where R = mycelium length per unit of soil weight; A = area of the Petri dish; N = number of hyphal-line intersections; and H = total length of the hyphal-lines in the Petri dish (cm).

The incidence of CLR was determined by the percentage of symptomatic leaves. For this, the number of diseased leaves was divided by the total number of leaves in each evaluated coffee plant. Then, the value obtained was multiplied by 100 to determine the percentage of incidence of CLR. To measure severity, a scale designed by Julca et al. (2019) was used and that considers the level of damage to the leaf area, which ranges from 0 to 80 % (Figure 1). The severity of each leaf was evaluated in each coffee plant. Then, the total average was taken for reporting the severity of CLR infection of the plant.

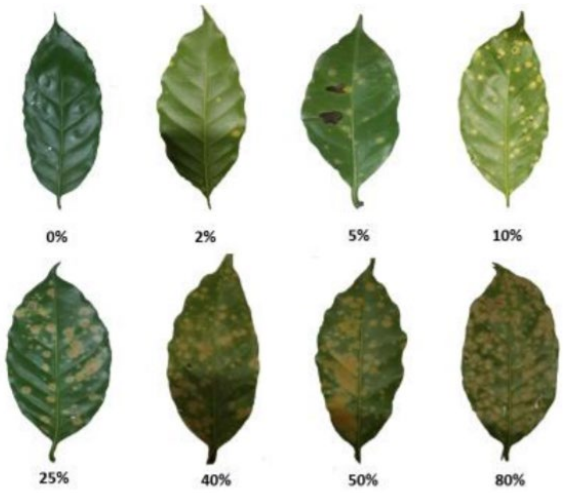

Figure 1. Scale used to quantify the severity of coffee leaf rust on *Coffea arabica* (Julca et al., 2019)

Data analysis. This study used a randomized complete block design. The Shapiro-Wilk test (at 0.05) was applied to contrast the normality of the data, before performing the analysis of variance. Inferences were obtained by analysis of variance and means comparison test (Tukey, $P \leq 0.05$) using the programming language with a focus on R statistical analysis and package agricolae.

Non-metric multidimensional scaling (NMDS) ordinations were used to show dissimilarities of coffee plants as influenced by the AMF inoculation, based on the Bray-Curtis distances, using the statistical software PAST, version 4.10.

RESULTS

The NMDS plot showed dissimilarities in the responses to coffee leaf rust infection. The first group corresponds to non-AMF coffee plants, while the second group includes plants inoculated with AMF (Figure 2). The factor AMF inocula showed significant effects on the extra-radical mycelium length, mycorrhizal colonization, plant height and number of branches; however, the coffee variety factor did not show significant statistical differences ($P \leq 0.05$) in any of the variables under study (Table 4). Moreover, there was no interaction between factors in the analysis of the variables evaluated, although it is important to highlight that the vegetative growth characteristics of both varieties Caturra and Pache were within the standard for the region in which this study was performed. Regarding the AMF factor, the Huallaga inoculum was superior to the other AMF inocula, promoting the obtaining of coffee plants 54.8 cm tall and with 13.2 branches per plant, on average. Even more, the results showed that the Huallaga inoculum achieved 64.3 % of mycorrhizal colonization and 46.7 cm of extra-radical mycelium length (Table 4). Contrary, the lowest morphological development results were found in the non-AMF treatment, in which smaller plants with yellowish leaves were observed.

The CLR incidence and severity data in coffee plants evaluated in the tenth month showed that both factors coffee variety and AMF have positive influences in the biological control of CLR. Coffee plants had a lower presence of CLR in January and May 2020 (dry season), while during the months October to December 2019 and June to July 2020 (rainy season) the incidence and severity of infection by CLR was higher.

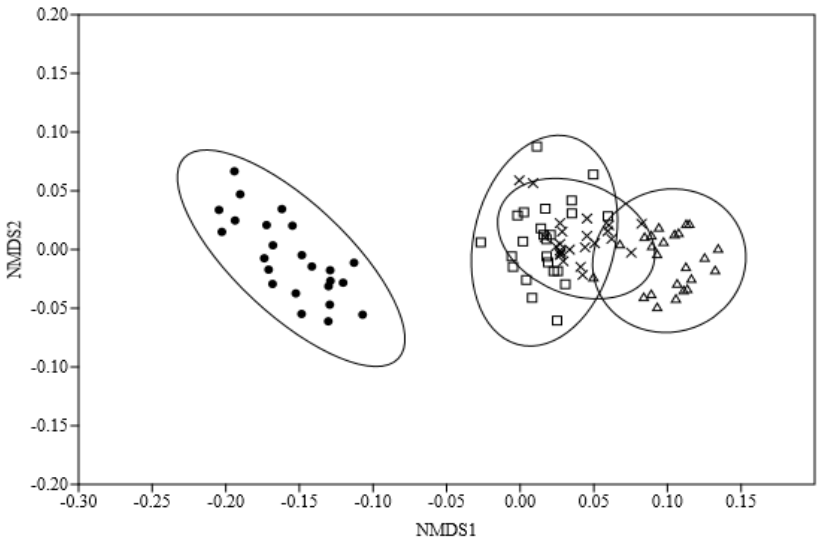

Figure 2. Non-metric multidimensional scaling ordination of coffee plants as influenced by the AMF inoculation. Stress = 0.1268. Non-AMF(●), Moyobamba inoculum (□), El Dorado inoculum (×) and Huallaga inoculum (Δ)

In the evaluation carried out 10 months after the installation of the coffee plants in the field, it was observed that the plants that were not inoculated with AMF had a higher incidence and severity of CLR infection (Figures 3 and 4). The variety Caturra was less tolerant to CLR, and showed higher incidence and severity of CLR in comparison to Pache variety. In Caturra-Non-AMF the incidence was 45.2 % and the severity equal to 37.4 %, while in the case of Pache-Non-AMF the incidence was 42.2 % and the severity equal to 32.2 %. In contrast, the Huallaga inoculum presented the lowest incidence and severity of CLR in the plants.

Table 4. AMF colonization and plant response according to coffee variety and inoculum type

	Extra-radical mycelium length (cm)	Colonization (%)	Plant height (cm)	Number of branches
Factor A: Coffee variety				
Caturra	36.8 a	54.5 a	45.9 a	11.7 a
Pache	38.9 a	53.9 a	41.7 a	11.1 a
Factor B: AMF				
Non-AMF	22.2 c	37.4 c	32.0 c	6.3 b
Moyobamba inoculum	39.9 b	52.4 b	41.1 b	13.0 a
El Dorado inoculum	42.5 ab	62.8 a	42.4 b	12.6 a
Huallaga inoculum	46.7 a	64.3 a	54.8 a	13.2 a

Means in the same column followed by different letters indicate significant differences among treatments according to Tukey test ($P \leq 0.05$).

It was found statistical significance between the coffee variety and AMF inoculum ($P \leq 0.05$) for both incidence and severity of CLR. For example, the Huallaga inoculum had better control of the incidence of CLR in Pache variety as compared to Caturra as shown in the different slope of the lines of non-AMF and Huallaga inoculum (Figure 3). Likewise, the inocula Moyobamba and El Dorado had different effect on severity of the disease (Figure 4).

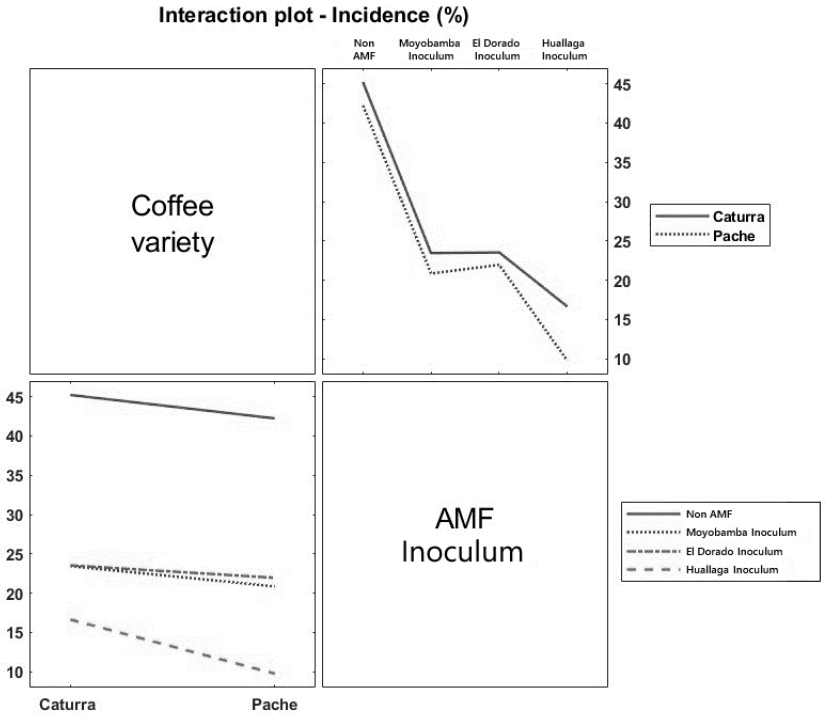

Figure 3. Interaction effect matrix plot: Incidence (%) as a dependent variable

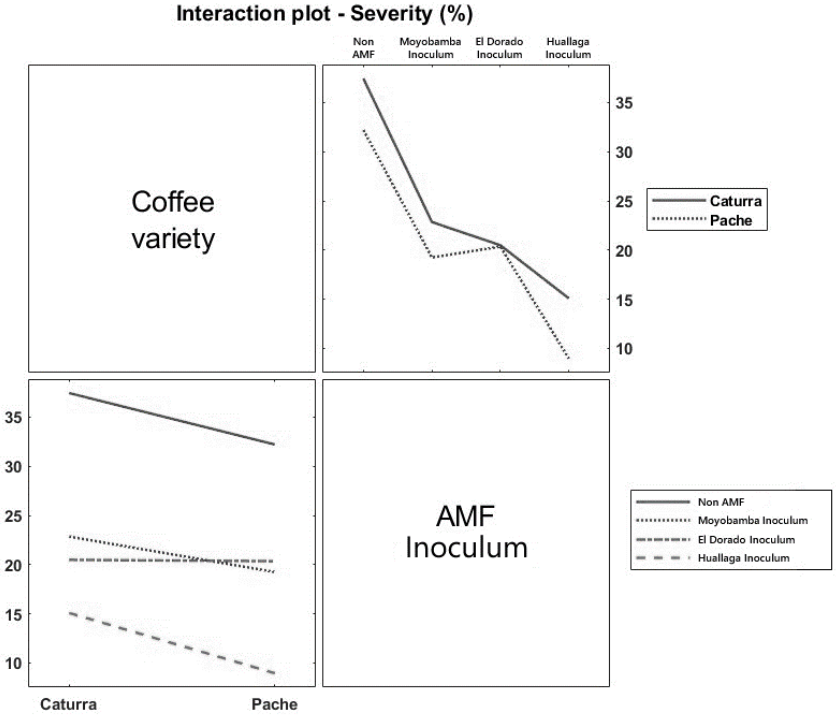

Figure 4. Interaction effect matrix plot: Severity (%) as a dependent variable

DISCUSSION

The symbiotic relationship that occurs between AMF and the root system of plants increases the absorption capacity of nutrients available in the soil causing greater vegetative development of plants, being the AMF benefited with carbon sources from the plants (Martín et al., 2010). Our study reports that the use of AMF improves the vegetative development of coffee plants. Huallaga inoculum favored the growth of tallest plants with higher number of branches, statistically superior to plants that were not inoculated with AMF. In the same line, Del Águila et al. (2018) reported that the use of AMF favors plant growth, in addition to inducing an increase in aerial biomass and dry root biomass of coffee plants.

Clonally propagated plants can photosynthesize and grow faster compared to plants propagated by botanical seeds (Boedeltje et al., 2008), so they may have a greater capacity to form symbiosis with AMF. Previous studies have shown that vegetatively propagated coffee plants have positive results for mycorrhizal colonization after AMF inoculation (Del Águila et al., 2018). As we mentioned, the AMF of Huallaga inoculum reached 64.3 % mycorrhizal colonization and 46.7 cm of extra-radical mycelium length, showing greater effectiveness when were compared with coffee plants that were not inoculated, which reached 37.4 % mycorrhizal colonization and 22.2 cm of mycelium length. Therefore, Huallaga inoculum of AMF may contribute to the proper functioning of agroecosystems, as well as helping in the recovery processes of degraded soils.

The CLR is a fungal disease that causes significant economic losses to coffee farmers worldwide. This disease is associated to the genotype, shade trees, rain, temperature, and load of fruits. Shade trees favor the infection, germination, and penetration of the uredospores into the leaves (Alvarado et al., 2020). This explains the reason why in our study the highest incidence and severity of CLR was higher during the rainy season. Furthermore, the study carried out in the Peruvian Amazon by Julca et al. (2019) found a positive correlation between the incidence and severity of CLR. Considering that the organic production of coffee in Peru has a growing trend, it is necessary to develop alternatives for the biological control of the diseases that affect this crop, among them CLR, which is the most devastating disease of coffee. In this context, the use of AMF represents a sustainable option against CLR.

The number of *H. vastatrix* spores per infected leaf area was significantly lower in coffee hybrids than in the Caturra inbred line (Toniutti et al., 2017) showing that the resistance of coffee plants to CLR is genetically controlled, but it can be affected by cultural practices if there is no proper management of the plantations (Dordas, 2008). Defense mechanisms in host plants are attenuated to facilitate root colonization with AMF (Chen et al., 2018), but then these mycorrhizal plants show greater tolerance to diseases and react faster to the pathogens attack (Borowicz, 2001). This phenomenon is known as induced systemic resistance.

AMF do not offer complete immunity against pathogens in host plants, but previous studies have reported that mycorrhizal plants often show greater resistance to disease attack (Chen et al., 2018; Jung et al., 2012). A study carried out in Mexico reported that the percentage of mycorrhizal colonization was higher in samples from coffee plants without signs of CLR infection (Herrera et al., 2019). Different fungi have been reported as potential agents for the biological control of spores of CLR. *Lecanicillium lecanii*, for example, is an entomopathogenic and myco-parasitic fungus that attacks spores of *H. vastatrix* in the field, showing an antagonistic effect (Vandermeer et al., 2009), but according to the report of Jackson et al. (2012) there is a one-year time lag relationship between outbreaks of the fungus and the low coffee leaf rust intensities. A bioassay performed in Costa Rica reported that *Simplicillium lanosoniveum* colonized coffee rust pustules (García and Hidalgo, 2020) showing great potential to be used as biological controller. In addition to these previous results, our study found that the application of AMF in vegetatively coffee plants prior to field establishment influenced in the reduction of the incidence and severity of CLR under field conditions.

The application of AMF should be complemented with other agricultural practices such as the installation of coffee plantations in agroforestry systems or the scheduled pruning of coffee trees, to enhance the effects of AMF. Moreover, monitoring the intensity and severity constitute a good practice for preventing CLR infections.

CONCLUSION

The application of arbuscular mycorrhizal fungi (AMF) increased the percentage of mycorrhizal colonization in roots of coffee plants compared to non-AMF plants. The NMDS analysis showed two well-defined groups in which all coffee plants inoculated with AMF were dissimilar to non-AMF coffee plants. The inoculation of AMF on vegetatively propagated coffee plants in the nursery stage reduced the incidence and severity of leaf rust, and also stimulated the vegetative growth of coffee plants. Therefore, the use of AMF may represent a promising option for the biological control of leaf rust in commercial coffee plantations.

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