ALTERNATIVES FOR THE MANAGEMENT OF COFFEE CORKY-ROOT DISEASE: SEARCH FOR TOLERANCE AND ANTAGONISTIC FUNGI †

[ALTERNATIVAS PARA EL MANEJO DE LA CORCHOSIS DE LA RAIZ DEL CAFETO: BUSQUEDA DE TOLERANCIA Y HONGOS ANTAGONISTAS]

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SUMMARY

Background: The root-knot nematode *Meloidogyne paranaensis* is one of the main phytosanitary problems in coffee crops, as it causes premature death of plants. In localities in central Veracruz, *Coffea canephora* plants tolerant to this nematode have been detected, which can be used as rootstocks in infested areas. **Objective**: To select plants that show tolerance in the field and to isolate and identify fungi associated with the rhizosphere of these coffee plants. **Methodology**: Plants infested with nematodes were selected and root samples were taken to quantify population density and corroborate the species. Fungi were isolated and identified from the rhizosphere soil. Five fungi were selected to observe the *in vitro* infection process on *M. paranaensis* eggs. **Results**: 18 plants that showed tolerance to *M. paranaensis* were selected. Twenty strains of fungi belonging to 7 genera and 16 species were obtained. The genera *Penicillium* and *Fusarium* were the most frequent. *Clonostachys rogersoniana* was the only fungus that showed parasitism against *M. paranaensis* eggs *in vitro*. **Implications**: The 18 selected plants show tolerance in the field, and it is necessary to conduct tests with controlled inoculations of *M. paranaensis* with clones and progeny. Tests on the nematophagous capacity of *C. rogersoniana* should continue. **Conclusion**: In the study area there are plants with tolerance to *M. paranaensis* and they can be used as rootstocks. The fungus *C. rogersoniana* is capable of infesting *M. paranaensis* eggs and is a potential biological control agent for this nematode.

Key words: *Meloidogyne paranaensis*; root-knot nematode; tolerant plants; nematophagous fungi; soil borne diseases.

RESUMEN

Antecedentes: El nematodo agallador *Meloidogyne paranaensis* es uno de los principales problemas fitosanitarios del cultivo de café ya que provoca la muerte prematura de las plantas. En localidades del centro

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de Veracruz se ha detectado plantas de *Coffea canephora* tolerantes a este nematodo, que pueden utilizarse como portainjertos en áreas infestadas. **Objetivo**: Seleccionar plantas que muestren tolerancia en campo y aislar e identificar hongos asociados a la rizosfera de estos cafetos. **Metodología**: Se seleccionaron plantas infestadas con nematodos y se tomaron muestras de raíz para cuantificar densidad de población y corroborar la especie. Se aislaron e identificaron hongos del suelo rizosférico. Se seleccionaron cinco hongos para observar el proceso de infección *in vitro* sobre huevos de *M. paranaensis*. **Resultados**: Se seleccionaron 18 plantas que presentaron tolerancia a *M. paranaensis*. Se obtuvieron 20 cepas de hongos pertenecientes a 7 géneros y 16 especies. Los géneros *Penicillium* y *Fusarium* fueron los más frecuentes. *Clonostachys rogersoniana* fue el único hongo que mostró parasitismo contra los huevos de *M. paranaensis in vitro*. **Implicaciones**: Las 18 plantas seleccionadas muestran tolerancia en campo y es necesario realizar pruebas con inoculaciones controladas de *M. paranaensis* con clones y progenie. Se debe continuar con las pruebas sobre la capacidad nematófaga de *C. rogersoniana*. **Conclusión**: En la zona de estudio hay plantas con tolerancia a *M. paranaensis* y que tienen posibilidad de utilizarse como portainjertos. El hongo *C. rogersoniana* es capaz de infestar huevos de *M. paranaensis*, y es un potencial agente de control biológico de este nematodo.

Palabras clave: *Meloidogyne paranaensis*; nematodos agallador; plantas tolerantes; hongos nematofagos; plagas del suelo.

INTRODUCTION

Coffee growing is one of the main economic activities for more than 60 countries in Latin America, Africa, and Asia. Approximately 120 million people depend directly or indirectly on its production, Brazil, Vietnam, Indonesia, and Colombia are the main producers (FAO, 2024). Mexico is the thirteenth largest producer with more than 180 thousand tons of green coffee harvested per year, by more than 500 thousand producers in 48 municipalities (FAO, 2024, SIAP, 2024). In 2023, almost 700 thousand hectares were planted, with the states of Chiapas and Veracruz being the main producing states, contributing 34.8 and 20.6% of the total national volume (SIAP, 2024). Currently one of the main phytosanitary problems affecting coffee production in America are the root-knot nematodes (Villain *et al*., 2013). These nematodes cause considerable economic losses by affecting the root and preventing the proper development of the plant (Elling, 2013; Villain *et al*., 2013). In particular, *Meloidogyne paranaensis* is the most harmful species for coffee plants as it is involved in the development of the disease known as coffee corky-root, which begins with the infestation of the nematode and gradually worsens with the subsequent arrival of phytopathogenic and opportunistic fungi and bacteria (López-Lima *et al*., 2020; Lamelas *et al*., 2020). Infested plants have reduced longevity, forcing growers to constantly replace plantations (Lima-Rivera *et al*., 2024). In Mexico, there are records of damage caused by coffee corky-root disease since the 1960s, however, until the 2000s it was only distributed in small areas of the states of Veracruz and Chiapas (Vázquez, 1963; López-Lima, 2021). Since 2013, with the massive replacement of coffee plants due to the epidemic of yellow leaf rust (*Hemileia vastatrix*) (Chort and Berk, 2024), coffee corky-root disease was widely distributed because plants infested with *M. paranaensis* were planted in fields free of this nematode (López-Lima, 2021). To date, *M. paranaensis* is the only root-knot nematode species associated with the development of coffee corky-root disease in Mexico (López-Lima *et al*., 2015; Alcasio-Rangel *et al*., 2017). To mitigate this problem, *Coffea canephora* (robusta) rootstocks are used because the plants of this species have a more vigorous root system, which increases the longevity of the plants for some years. However, they are susceptible to infestation by *M. paranaensis* and represent a temporary solution, since most of the grafted plants end up dying prematurely (Fatobene *et al*., 2019). The oldest records of the presence of coffee corky-root disease in Mexico come from the central mountainous area of Veracruz, in the municipalities of Tlaltetela and Totutla (Vázquez, 1963). In some localities of the Totutla municipality, farmers began planting robusta coffee trees in the 1980s in order to mitigate the effects of disease. In previous surveys, it was detected that some robusta coffee plants have shown good levels of development, vigor, and longevity despite being affected by coffee corky-root disease. The study of these plants could generate alternatives to increase the longevity of plantations in infested areas. Since in Mexico there are no coffee varieties with tolerance to *M. paranaensis* that are used as rootstocks and no studies have been conducted on microorganisms antagonistic to *M. paranaensis*, the objective of this work was to carry out a first selection of plants that show tolerance in the field and to isolate and identify fungi associated with the rhizosphere of these coffee plants with the potential to be used as biological control agents for *M. paranaensis*.

MATERIALS AND METHODS

Study site and plant selection

Sampling was conducted in a variable width transect through the coffee plantation area of the towns of Mata Obscura and Mata de Indio in the municipality of Totutla. The plant selection criteria were based on the age (10 years minimum), presence of coffee corky-root symptoms and plant vigor (Cerda-Ocaranza *et al*., 2023). Root and rhizospheric soil samples were taken from the selected plants, which were placed in polypropylene bags and transported to the laboratory. The roots were washed with running water and dried with absorbent paper to obtain the fresh weight, observe the formation of galls or corky-root symptoms and assign a number on the galling scale (Coyne and Rose, 2014). Subsequently, the roots were cut into 1-2 cm fragments and 20 g were taken to extract nematode eggs and second stage juveniles (J2) by sodium hypochlorite grinding, sieving and centrifugation technique (Carneiro *et al*., 2004). A 10 mL suspension of eggs and J2 was obtained from which three one mL aliquots were taken and counted in a Sedgwick-Rafter chamber under an optical microscope at 100X. Population density (eggs and J2 g root^{-1}) was calculated by dividing the average nematode count by the weight of the root processed for extraction (average nematode count/weight of processed root). The identification of the nematodes obtained from the roots was corroborated by the *M. paranaensis* species-specific SCAR marker (Randig *et al*., 2002).

Isolation and identification of fungi

Isolation of fungi from rhizospheric soil was performed by the agar plate dilution method (Carrion *et al*., 2021). Five g of soil were taken from each sample and diluted in 10 mL of sterile water. The solution was vortexed for five minutes. 100 µL of this solution was then inoculated into Petri dishes (80 mm diameter) with potato dextrose agar (PDA) culture medium. The Petri dishes were incubated at 22 ºC and observed daily. Fungal colonies that grew on the agar plates were separated into new PDA dishes until pure cultures were obtained.

From the pure cultures of each fungus, DNA was extracted using the Kit Zymo Research Quick-DNA Fungal/Bacterial Miniprep Kit ®. A 500 bp molecular marker that encompasses the Internal Transcriber Spacer (ITS) 1 and the ITS2 molecular markers, were used and amplified by PCR (Schoch *et al*., 2012). The PCR products were analyzed on a 1.2 % agarose gel; and the DNA was purified and sent to Macrogen INC for sequencing. The obtained sequences were edited in the FinchTV 1.4.0 program and compared by BLAST analysis to the database of the National Center for Biotechnology Information (NCBI).

Fungal infection process on *M. paranaensis* eggs

To make a first selection of strains with nematicidal potential, an *in vitro* test was made exposing viable eggs of *M. paranaensis* to five fungi, selected based on their identification and the genus history of use as biological control agents. Nematode eggs were extracted by the method of grinding in sodium hypochlorite, sieving and centrifuging from *C. arabica* roots previously infested with *M. paranaensis* (Carneiro *et al*., 2004). The extraction solution was quantified in a Sedgwick-Rafter counting chamber and adjusted to 1000 eggs mL^{-1}. Subsequently, 100 µL of the *M. paranaensis* egg suspension was placed in a Petri dish with a previously prepared agar-water plate. In parallel, a suspension of 1x10^{6} spores mL^{-1} of each selected fungus was made with sterile distilled water and 100 µL of this solution was placed on the eggs previously deposited on the agar-water plate. To observe the infection process, spore germination was stopped every 24 hours on five occasions by adding a drop of lactophenol blue. The eggs were then placed on a slide to observe under a microscope characteristics such as germination or penetration of the hyphae into the cuticle of the eggs. This procedure was performed in quintuplicate for each fungal strain and for each hour of sampling. Likewise, a group of eggs were kept without inoculation as an untreated control (N=150).

RESULTS AND DISCUSSION

Coffea canephora plants with tolerance to *Meloidogyne paranaensis* selected *in situ*

Eighteen *C. canephora* plants distributed in 9 farms were selected (Table 1). The plants showed good development with abundant shoot generation despite the infestation (Figure 1A). Young roots exhibited gall formation (Figure 1B), and woody roots showed symptoms of corky-root such as thickening of the cortical tissue, necrosis (Figure 1C) and the presence of numerous *Meloidogyne* female embedded in the tissue (Figure 1D). The galling level of the 18 plants was light to medium, at levels 2 and 3 on the scale, and the averaged population density of 79.3 eggs and J2 g root^{-1}

(Table 1). The use of nematode-tolerant plants is one of the most effective nematode management strategies, since, in most cases, these plants maintain their level of productivity even when they are infested (Shigueoka *et al*., 2016). Likewise, microorganisms present in the rhizosphere can function as regulators of pest populations, giving the plant a chance of overcoming the infestation (Topalovic *et al*., 2020). In Mexico there are no *C. canephora* selections or cultivars with proven resistance or tolerance to *M. paranaensis* that can be used systematically as rootstocks. The search for tolerant plants in infested areas is a commonly strategy, however, further studies are needed with the plants selected in this work, in order to determine the origin of the tolerance observed in the field, which may be due, in addition to genetic factors, to the edaphoclimatic conditions of the site or the presence of microorganisms antagonistic to nematodes in the rhizosphere (Sera *et al*., 2010; Lima-Salgado *et al*., 2014).

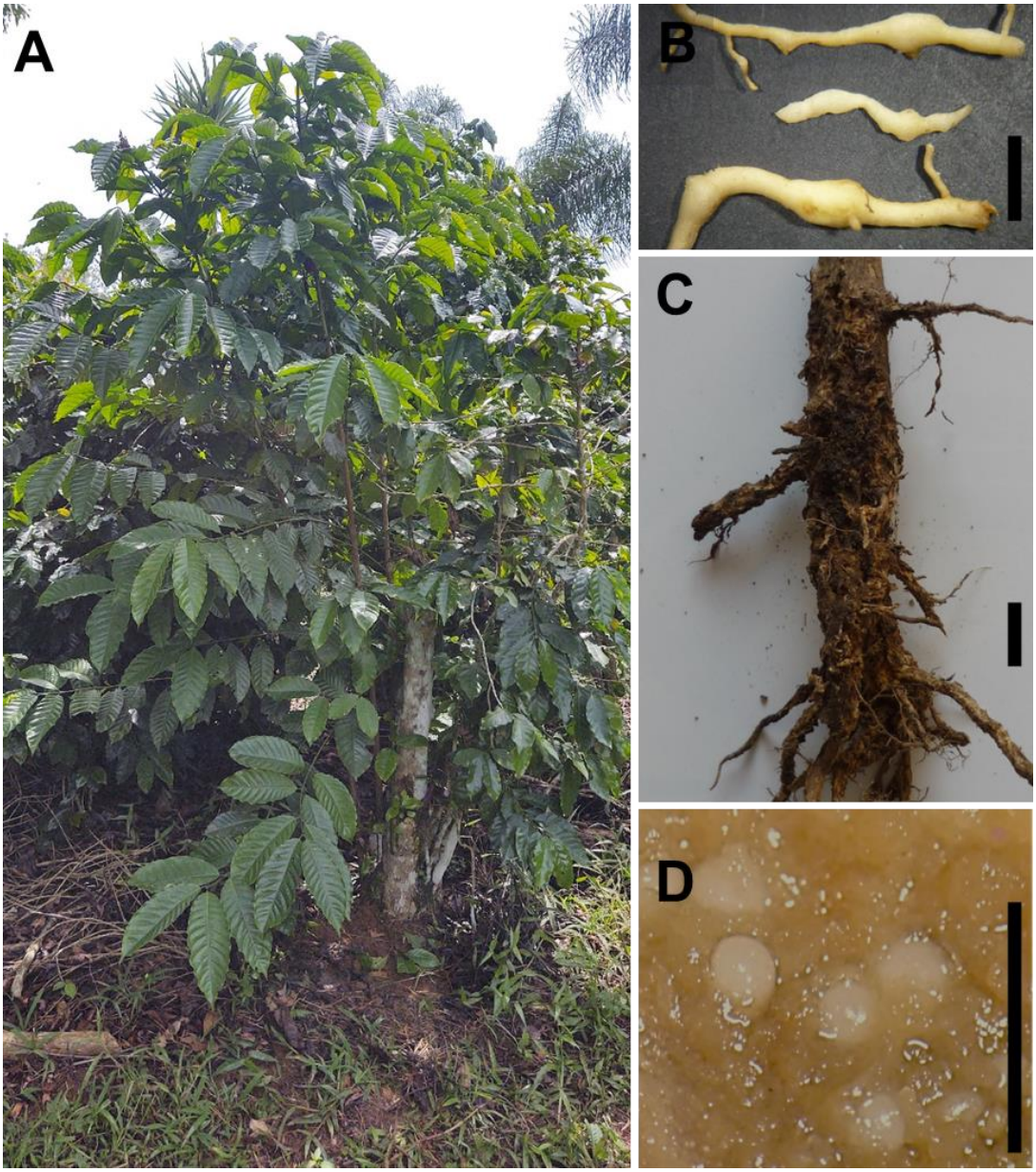

Figure 1. *Coffea canephora* plant infested with *Meloidogyne paranaensis*. A) Aerial aspect of the infested plant showing vigor and development, B) secondary roots with thickening caused by *M. paranaensis*, C) Woody root with symptoms of corchosis, D) *M. paranaensis* females embedded in the corky tissue 40X. Barr = 1 cm.

Table 1. Geographic data of sampling sites, galling level, and population density of *Meloidogyne paranaensis* of the selected plants (average of two plants).

Locality	Site	Location	Altitude (masl)	Galling index	Population density eggs and J2 g root^{-1}
Mata	1	19º13'17.0"N; 96º51'08.3"W	947	2	58
oscura	2	19º14'03.9"N; 96º50'33.2"W	896	2.5	88.5
	3	19º13'30.9"N; 96º51'07.4"W	939	3	106
	4	19º13'20.4"N; 96º51'16.2"W	957	2.5	84
Mata	5	19º14'01.0"N; 96º50'36.2"W	898	2	74.5
de	6	19º13'48.1"N; 96º50'52.6"W	922	2	74.5
Indio	7	19º13'37.6"N; 96º50'46.9"W	921	2.5	79.5
	8	19º14'02.7"N; 96º50'26.6"W	889	2	73
	9	19º13'57.8"N; 96º50'32.2"W	896	2	76

Native fungi associated with the rhizosphere of *Coffea canephora* with corky-root disease

Twenty fungal strains belonging to 7 genera and 16 species were isolated from the sampled sites. The genus *Penicillium* was the most frequent with seven isolates followed by *Fusarium* with six isolates. The most frequent species was *Fusarium oxysporum* with four isolates (Table 2).

Many fungi have been reported associated with coffee plants, the main fungal families recorded are Aspergillaceae and Netriaceae (Lu *et al*., 2022), which coincides with the results of this work where the most frequent species belong to these families. Likewise, in a study of the rhizosphere microbiome of coffee plants, the genera *Fusarium*, *Trichoderma*, *Aspergillus* and *Rhizophagus* were the most abundant (de Sousa *et al*., 2022). In another study, fungi were isolated directly from coffee corky-root tissues, and 49 isolates were obtained, *Fusarium* and *Penicillium* were the main genera with a relative abundance of 65 and 10% respectively (Lopez-Lima *et al*., 2020). Many of the fungi found in this work have reports of biological activity against pest nematodes, for example, *Trichoderma spirale* has been reported parasitizing *Radopholus similis*, *Meloydogyne exigua* and *Meloidogyne incognita* (Varela-Benavides *et al*., 2017). *Penicillium* species are

Table 2. Molecular identification of fungal species isolated from coffe rhizosohere of plants with corky-root disease.

Locality	Site	Fungi species molecular diagnosis	NCBI accession number	% identity
Mata oscura	1	*Penicillium tanzanicum*	NR_158820.1	100%
		Penicillium glaucoroseum	MT530148.1	99.8%
		Fusarium proliferatum	OQ363325.1	100%
	2	*Fusarium oxysporum*	PP596543.1	100%
	3	*Fusarium oxysporum*	OQ683854.1	100%
		Penicillium tealli	OP101644.1	100%
		Trichoderma kaningiopsis	MT520626.1	100%
	4	*Penicillium jiangxiense*	KP204361.1	100%
Mata de	5	*Trichoderma spirale*	KF624809.1	100%
Indio		*Gleotinia temulenta*	KT876537.1	100%
		Penicillium kloeckeri	KT385771.1	98.9%
	6	*Clonostachys rogersoniana*	MH421856.1	100%
		Penicillium glaucoroseum	MT530148.1	100%
	7	*Aspergillus westerdijkiale*	MT378420.1	100%
		Fusarium oxysporum	PP596543.1	100%
	8	*Metarhizium marquandii*	MH483861.1	100%
		Fusarium graminearum	PP620750.1	100%
		Fusarium oxysporum	PP596543.1	100%
		Penicillum stratisporum	OQ332398.1	100%
	9	*Metarhizium robertsii*	MT378171.1	99.8%

widely used as biological control agents, in particular nematicidal activity has been reported in *P. commune* and *P. chrysogenum* species (Sikandar *et al*., 2020; Nguyen *et al*., 2021). Non-pathogenic strains of *Fusarium oxysporum* have been found parasitizing *R. similis*, *Pratylenchus goodey* and *M. incognita* (Dababat and Sikora, 2007; Van Dessel *et al*., 2011). However, pathogenic strains of *F. oxysporum* are commonly associated with coffee corky-root and this fungus is considered one of the main causative agents of this disease (López-Lima *et al*., 2020), which may explain the frequency isolation of *F. oxysporum* in this work. On the other hand, the fungus *Metarhizium robertsii* has been reported for use in biological control of fungi and insects, for example, it is antagonistic to the phytopathogenic fungus *Cochliobolus heterostrophus* and pathogenic to the insect pest *Spodoptera frugiperda* (Flonc *et al*., 2021; Ahmad *et al*., 2022).

In vitro fungal infection process on *M. paranaensis*

The eggs infection process was conducted with the *Aspergillus westerdijkiae*, *Clonostachys rogersoniana*, *Gleotinia temulenta*, *Penicillium glaucoroseum* and *Penicillium striatisporum* strains. For *A. westerdijkiae*, no spore germination was observed in the first 24 and 48 h, at 72 and 96 h the development of hyphae was observed on the outside of the eggs and at 120 h the presence of hyphae surrounding the cuticle of the eggs was observed, but with no evidence of penetration (Figure 2A). For *C. rogersoniana*, no spore germination was observed in the first 24 h, at 48 h spore germination was observed on the cuticle of the eggs, at 72 h the eggs were found surrounded by mycelium and for 96 and 120 h the mycelium was observed inside the eggs and the degradation of the content (Figure 2B). In the eggs treated with *G. temulenta*, no spore germination or mycelium was observed in any of the observations (Figure 2C). No germination of *P. glaucoroseum* spores was observed at 24 and 48 h. At 72 and 96 h, hyphal development was observed and at 120 h abundant mycelial development was seen around the eggs with no evidence of penetration (Figure 2D). Spores of *P. stratisporum* were observed attached to the eggs, but no evidence of germination during the experiment (Figure 2E). Eggs without the application of fungus remained without mycelial

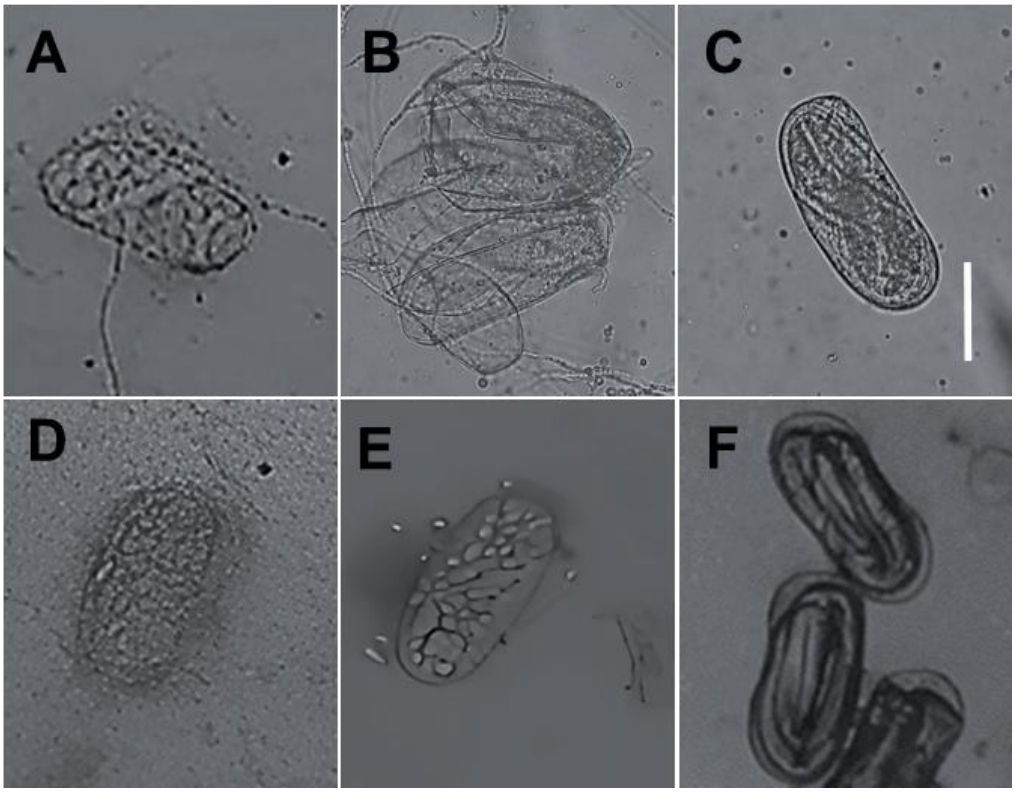

Figure 2. Infection process of fungi isolated from the rhizosphere of *Coffea canephora* on *Meloidogyne paranaensis* eggs at 120 h after inoculation. A) *Aspergillus westerdijkiae*, B) *Clonostachis rogersoniana*, C) *Gleotinia temulenta*, D) *Penicillium glaucoroseum*, D) *Penicillium striatisporum* and F) untreated control. 400 X. Bar = 30 µm.

development or alterations during the experiment (Figure 2F). *Aspergillus* strains have demonstrated nematicidal activity, for example, *Aspergillus* sp. strain SPH2 kills 23.7% of *M. javanica* juveniles under *in vitro* conditions (Morales-Sánchez *et al*., 2021). *Aspergillus fumigatus* produces 2-furoic acid in liquid fermentation and exposure to the culture broth causes mortality of J2 juveniles of *M. incognita* at a dose of 37.75 µg/mL (Wang *et al*., 2022). Likewise, the fermentation filtrate of *Aspergillus japonicus* kills 100% of the J2 juveniles of *M. incognita* within 24 h and inhibits the eggs hatching (He *et al*., 2020). It is necessary to evaluate the nematicidal activity of the fermentation metabolites of *A. westerdijkiae*, since in this work with the direct application of spores no penetration of mycelium into the eggs was observed. The *Clonostachys* genus is widely recognized as a biological control agent for its production of secondary metabolites that have pharmaceutical or agrochemical applications (Han *et al*., 2020). In particular, *C. rogersoniana* is known to produce verticillins, which are dimeric epipolythiodixopiperazines with antimicrobial activity (Guo *et al*., 2017). *Clonostachys rogersoniana* was the only fungus that showed egg degradation, tests are needed to determine its potential use as a biological control agent for nematodes.

The nematicidal potential of *Penicillium* strains has been previously demonstrated. For example, the fermentation broth of *Penicillium chrysogenum* causes up to 97% mortality of J2 juveniles and 94% inhibition of hatching after 72 h of exposure to *M. incognita* eggs (Sikandar *et al*., 2020). The nematicidal activity of *Penicillium* is related to secondary metabolites production, as studied in *Penicillium commune*, which produces cyclopiazonic acid and has activity against second-stage juveniles of *M. incognita*, *M. hapla* and *M. arearia* (Nguyen *et al*., 2021). The two *Penicillium* species evaluated in this work did not show penetration of the egg cuticle by hyphae, however, it is necessary to study the effect of their fermentation metabolites.

The data obtained in this work do not allow us to determine whether the tolerance shown by the *C. canephora* plants at the study site is due to the presence of certain fungal species, since no fungal species was detected in common for all sites. It is necessary to perform nematicidal activity tests with all the strains isolated individually and in consortia by isolation sites in order to determine whether these influence the tolerance to *M. paranaensis* shown by the plants.

CONCLUSION

The use of different alternatives to mitigate the *M. paranaensis* population is the best strategy to reduce the effects of coffee corky-root disease. The sowing of plants with tolerant rootstocks can be complemented with the application of antagonistic organisms and even with the application of synthetic nematicides at crucial moments such as the establishment of new plants. This work shows that in the study area there are plants that can tolerate the infestation by *M. paranaensis* and that have the possibility of being used as rootstocks. It is necessary to conduct studies with clones and progeny of the selected plants in controlled inoculations with different population densities of *M. paranaensis* to determine their tolerance level. Likewise, the evaluation of the fungus *C. rogersoniana* should continue since this work demonstrated its ability to infest *M. paranaensis* eggs and be used as a biological control agent for this nematode.

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Compliance with ethical standards. The authors confirm that this investigation was conducted under the current ethical procedures. No humans or animals were used in the studies of this article.

Data availability. Data is available with the corresponding author upon request.

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REFERENCES

Alcasio-Rangel, S., Torres-López, J. and López-Buenfil, J.A., 2017. Detecciones del nematodo agallador *Meloidogyne paranaensis* en Puebla y Veracruz, México. *Revista Mexicana de Fitopatología*, 35, pp. S65.

Ahmad, I., Jiménez-Gasco, M. d. M., Luthe, D.S. and Barbercheck, M.E. 2022. Endophytic *Metarhizium robertsii* suppresses the phytopathogen, *Cochliobolus heterostrophus* and modulates maize defenses. *PLoS ONE*, 17, pp. e0272944. https://doi.org/10.1371/journal.pone.0272944

Carneiro, R.M.D.G., Tigano, M., Randig, O., Almeida, M.R. and Sarah, J.L., 2004. Identification and genetic diversity of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) on coffee from Brazil, Central America, and Hawaii. *Nematology*, 6, pp. 287-298. https://doi.org/10.1163/156854104121794242

Cerda-Ocaranza, M.G., Salgado-Mora, M.G., Aguirre-Cadena, J.F., and Chilel-Pérez, N.D. 2023. Respuesta del café robusta (*Coffea canephora*) Pierre ex A. Froehner a diferentes manejos de sombra y fertilización, en el sur de Chiapas, México. *Ciencia Latina Revista Científica Multidisciplinar*, 7(1), 5547-5565. https://doi.org/10.37811/cl_rcm.v7i1.4870

Carrión, G., López-Lima, D. and Durán-Barradas, Z., 2021. Hongos entomopatógenos, micoparásitos y nematófagos. In: G. Mata and D. Salmones eds. *Técnicas de aislamiento, cultivo y conservación de cepas de hongos en el laboratorio*. Xalapa, México: Instituto de Ecología, A.C. pp. 73-86.

Chort, I. and Berk, Ö., 2024. Agricultural shocks, coping policies and deforestation: evidence from the coffee leaf rust epidemic in Mexico. *American Journal of Agricultural Economics* 106, pp. 1020–57. https://doi.org/10.1111/ajae.124411054

Coyne, D.L. and Ross, J.L., 2014. *Protocol for nematode resistance screening: root knot nematodes Meloidogyne spp*. Ibadan: International Institute of Tropical Agriculture (IITA).

Dababat, A. A. and Sikora, R. A., 2007. Induced resistance by the multualistic endophyte, *Fusarium oxysporum* strain 162, toward *Meloidogyne incognita* on tomato. *Biocontrol Science and Technology*, 17, pp. 969–975. https://doi.org/10.1080/09583150701582057

de Sousa, L.P., Guerreiro-Filho, O. and Mondego, J.M.C. 2022. The rhizosphere microbiomes of five species of coffee trees. *Microbiology Spectrum*, 10, e00444-22. https://doi.org/10.1128/spectrum.00444-22

Elling, A. A., 2013. Major emerging problems with minor *Meloidogyne* species. *Phytopathology*, 103, pp. 1092–1102. https://doi.org/10.1094/PHYTO-01-13-0019-RVW

FAO (Food and Agriculture Organization of the United Nations), 2024. FAOSTATS. Accessed date September, 12, 2024. https://www.fao.org/faostat/en/#data

Fatobene, B.J.R., Andrade, V.T., Gonçalves, W. and Guerreiro Filho, O., 2019. *Coffea canephora* clones with multiple resistance to *Meloidogyne incognita* and *M. paranaensis*. *Experimental agriculture*, 55, pp. 443-451. https://doi.org/10.1017/S0014479718000108

Flonc, B., Barbercheck, M. and Ahmad, I., 2021. Observations on the relationships between endophytic *Metarhizium robertsii*, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), and maize. *Pathogens*, 10, pp. 713. https://doi.org/10.3390/pathogens10060713

Guo, Z., Hao, T., Wang, Y., Pan, Y., Ren, F., Liu, X., Che, Y. and Liu, G., 2017. VerZ, a

Zn(II)2Cys6 DNA-binding protein, regulates the biosynthesis of verticillin in *Clonostachys rogersoniana*. *Microbiology*, 163, pp. 1654–1663. https://doi.org/10.1099/mic.0.000557

Han, P., Zhang, X., Xu, D., Zhang, B., Lai, D. and Zhou, L., 2020. Metabolites from *Clonostachys* fungi and their biological activities. *Journal of Fungi*. 6, pp. 229. https://doi.org/10.3390/jof6040229

He, Q., Wang, D., Li, B., Maqsood, A. and Wu, H., 2020. Nematicidal evaluation and active compounds isolation of *Aspergillus japonicus* ZW1 against root-knot nematodes *Meloidogyne incognita*. *Agronomy*, 10, pp. 1222. https://doi.org/10.3390/agronomy10091222

Lamelas, A., Desgarennes, D., López-Lima, D., Villain, L., Alonso-Sánchez, A., Artacho, A., Latorre, A., Moya, A., and Carrion, G., (2020) The bacterial microbiome of *Meloidogyne*-based disease complex in coffee and tomato. *Frontiers in Plant Science*, 11. https://doi.org/10.3389/fpls.2020.00136

Lima-Rivera, D., Anell-Mendoza, M.B., Rivera-Fernández, A., Salinas-Castro, A., Cerdán, C., López-Lima, D. and Villain, L., 2024. Host status of plants associated to coffee shady agroecosystems to *Meloidogyne paranaensis*. *Journal of Plant Disease and Protection*, 131, pp 873-880. https://doi.org/10.1007/s41348-024-00882-5

Lima-Salgado, S.M., Costa de Rezende, J. and Rodrigues-Nunes, J.A., 2014. Selection of coffee progenies for resistance to nematode *Meloidogyne paranaensis* in infested area. *Crop Breeding and Applied Biotechnology*, 14, pp. 94-101. https://doi.org/10.1590/1984-70332014v14n2a17

López-Lima, D., 2021. Determinación de los patógenos asociados a la corchosis de la raíz del cafeto en Veracruz. In: R. Zetina-Lezama, H. Tosquy-Valle, A.L. Del Ángel-Pérez, A. Ríos-Utrera, V. Vázquez-Hernández, V.A. Esqueda-Esquivel and C. Perdomo-Montes eds. *Contribuciones tecnológicas para el futuro forestal y agropecuario veracruzano 2021*. Ciudad de México: INIFAP. pp. 470-481.

López-Lima, D., Carrion, G., Sánchez-Nava, P., Desgarennes, D., and Villain, L., 2020. Fungal diversity and *Fusarium oxysporum* pathogenicity associated with coffee corky-root disease in México. *Rev. FCA Uncuyo*, 52, pp. 276-292. http://revistas.uncu.edu.ar/ojs3/index.php/RFCA/article/view/3075

Lopez-Lima, D., Sánchez-Nava, P., Carrión, G., Espinosa de los Monteros, A. and Villain, L. 2015. Corky-root symptoms for coffee in central Veracruz are linked to the root-knot nematode *Meloidogyne paranaensis*, a new report for Mexico. *European Journal of Plant Pathology*, 141, pp. 623-629 https://doi.org/10.1007/s10658-014-0564-9

Lu, L., Tibpromma, S., Karunarathna, S.C., Jayawardena, R.S., Lumyong, S., Xu, J. and Hyde, K.D., 2022, Comprehensive review of fungi on coffee. *Pathogens*. 11, pp. 411. https://doi.org/10.3390/pathogens11040411

Muller, G., Bills, G. and Foster, M., 2004. *Biodiversity of fungi: inventory and monitoring methods*. Londres: *Elsevier Academic Press*.

Morales-Sánchez, V., Díaz, C. E., Trujillo, E., Olmeda, S. A., Valcarcel, F., Muñoz, R., Andrés, M. F., and González-Coloma, A., 2021. Bioactive metabolites from the endophytic fungus *Aspergillus* sp. SPH2. *Journal of Fungi*, 7, pp.109. https://doi.org/10.3390/jof7020109

Nguyen, V. T. Yu, N. H. Lee, Y., Hwang, I. M., Bui, H. X. and Kim, J. C., 2021. Nematicidal activity of cyclopiazonic acid derived from *Penicillium commune* against root-knot nematodes and optimization of the culture fermentation process. *Frontiers in Microbiology*, 12, https://doi.org/10.3389/fmicb.2021.726504

Randig, O., Bongiovanni, M., Carneiro, R. and Castagnone-Sereno, P., 2002. Genetic diversity of root-knot nematodes from

Brazil and development of SCAR markers specific for the coffee-damaging species. *Genome,* 45, pp. 862–870. https://doi.org/10.1139/g02-054

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W. and Fungal Barcoding Consortium, 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *PNAS*, 109, pp. 6241-6246. https://doi.org/10.1073/pnas.1117018109

Sera, G.H., Sera, T., Fonseca, I.C.B. and Ito, D.S., 2010. Resistência à ferrugem alaranjada em cultivares de café. *Coffee Science*, 5, pp. 59-66. https://coffeescience.ufla.br/index.php/Coffeescience/article/view/272

SIAP (Servicio de Información Agroalimentaria y Pesquera), 2024. Anuario Estadístico de la Producción Agrícola 2023. Accessed date September, 12, 2024. https://nube.siap.gob.mx/cierreagricola/

Sikandar, A., Zhang, M., Wang, Y., Zhu, X., Liu, X., Fan, H., Xua, Y., Chen, L. and Duan, Y., 2020. *In vitro* evaluation of *Penicillium chrysogenum* Snef1216 against *Meloidogyne incognita* (root-knot nematode). *Scientific Reports*, 10, pp. 8342. https://doi.org/10.1038/s41598-020-65262-z

Shigueoka, L.H., Sera, G.H., Sera, T., Fonseca, I.C.B., Andreazi, E., Carvalho, F.G., Carducci, F.C. and Ito, D.S., 2016. Reaction of Arabica coffe progenies derivate from Icatu to *Meloidogyne paranaensis*. *Plant Protection* 75, 193-198. http://dx.doi.org/10.1590/1678-4499.229

Topalović, O., Hussain, M. and Heuer, H., 2020. Plants and Associated Soil Microbiota Cooperatively Suppress Plant-Parasitic Nematodes. *Frontiers in Microbiology*, 11, p. 313. https://doi.org/10.3389/fmicb.2020.00313

Van Dessel, P., Coyne, D., Dubois, T., De Waele, D. and Franco, J., 2011. *In vitro* nematicidal effect of endophytic *Fusarium oxysporum* against *Radopholus similis*, *Pratylenchus goodeyi* and *Helicotylenchus multicinctus*. *Nematropica*, 41, pp. 154-160. https://journals.flvc.org/nematropica/article/view/76618/74228

Varela-Benavides, I., Durán-Mora, J. and Guzmán-Hernández, T., 2017. Evaluación *in vitro* de diez cepas de hongos nematófagos para el control de *Meloidogyne exigua*, *Meloidogyne incognita* y *Radopholus similis*. *Tecnología en Marcha*, 30, pp. 27-37. https://doi.org/10.18845/tm.v30i1.3062

Vázquez, J.T., 1963. Relaciones suelo-nemátodos. In: *Memoria del 1er. Congreso Nacional de Ciencia del Suelo*. Mexico: Sociedad Mexicana de Ciencia del Suelo. pp. 201-203.

Villain, L., Sarah, J. L., Hernández, A., Bertrand, B., Anthony, F., Lashermes, P., Charmetant, P., Anzueto, F., Figueroa, P. and Carneiro, R.M.D.G., 2013. Diversity of root-knot nematodes parasitizing coffee in Central America. *Nematropica*, 43, pp. 194–206. https://journals.flvc.org/nematropica/article/view/82708

Wang, L., Qin, Y., Fan, Z., Gao, K., Zhan, J., Xing, R., Liu, S. and Li, P., 2022. Novel lead compound discovery from *Aspergillus fumigatus* 1T-2 against *Meloidogyne incognita* based on a chemical ecology study. *Journal of Agricultural and Food Chemistry*, *70*, pp. 4644-4657. https://doi.org/10.1021/acs.jafc.1c08147