ARBUSCULAR MYCORRHIZAL FUNGI IDENTIFICATION IN AVOCADO TREES INFECTED WITH *Phytophthora cinnamomi* RANDS UNDER BIOCONTROL

[IDENTIFICACIÓN DE HONGOS MICORRIZÓGENOS ARBUSCULARES EN ÁRBOLES DE AGUACATE INFECTADOS CON *Phytophthora cinnamomi* RANDS BAJO CONTROL BIOLÓGICO]

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SUMMARY

Arbuscular mycorrhizal fungi presences in the rhizosphere of avocado trees with symptoms of root rot sadness caused by *Phytophthora cinnamomi* were determined. The investigation was done in the avocado orchard "Ojo de agua" in the town of Tancitaro, Michoacan, Mexico, in 21 previously selected trees. For the control of *P. cinnamomi* were applied three strains of *Trichoderma* (*T. erinaceum*, *T. aggressivum* and *T. arundinaceum*) before the application was made the first soil sampling, the second 6 months later, before the second application of *Tricoderma* strains and the last 12 months before the third application. To remove soil spores was used wet sieving and decantation protocol proposed by Gerdemann and Nicolson (1963), followed by centrifugation on sucrose (400 g L-1) at 2000 rpm. Taxonomic identification was based on the morphological characteristics of AMF spores, considering the shape, size and color, and thickness, ornamentations and number of the layers of the wall, coupling form and supporting hyphae, identifications were made by comparison with original descriptions available in the International Collection of Arbuscular Mycorrhizal Fungi and Glomeromycota species list. The first sampling were identified eleven species in seven genera: *Glomus* with two undetermined species, *Glomus* sp.1, *Glomus* sp.2, *Glomus etunicatum* and *Glomus geosporum*; genus *Acaulospora*, one undetermined species *Acaulospora* sp., *A. spinosa*, *A. bireticulata* and *A. denticulate*; genus *Entrophospora*, *E. infrequens*; genus *Diversispora*, *D. aurantia;* genus *Scutellospora*, *S. pellucida;* genus *Racocetra*, *R. castanea* and *R. verrucosa* and genus *Gigaspora*, *Gi. decipiens*. In the second and third sampling, the presence of new kinds of HMA there was not observed but the number of spores increased (average 38.09% and 30% respectively). The application of these species in the genus *Trichoderma* to control root pathogens of avocado encouraged the growth of HMA spores in the rhizosphere of the crop.

Key words: *Phytophthora cinnamomi*; *Trichoderma*; avocado; biological control.

RESUMEN

Se determinó la presencia de hongos micorrizógenos arbusculares en la rizósfera de árboles de aguacate con síntomas de pudrición radicular tristeza, ocasionada por *Phytophthora cinnamomi*. El trabajo se realizó en el huerto de aguacate "Ojo de agua" en el municipio de Tancítaro, Michoacán, México, en 21 árboles previamente seleccionados. Para el control de *P. cinnamomi* se aplicaron tres especies de *Trichoderma* (*T. erinaceum*, *T. aggressivum* y *T. arundinaceum*) el primer muestreo se realizó previo a la aplicación, el segundo 6 meses después, antes de la segunda aplicación y el último a los 12 meses, antes de la tercera aplicación. Para extraer las esporas del suelo se utilizó el protocolo de tamizado húmedo y decantación propuesto por Gerdemann y Nicolson (1963), seguido de centrifugación en sacarosa (400 gL-1) a 2000 rpm. La identificación taxonómica fue con base en las descripciones originales, que consideran la forma, tamaño y color de las esporas; grosor, número y ornamentación de las capas de la pared, así como forma y acoplamiento de la hifa de sostén, se hicieron las identificaciones por comparación con las descripciones originales

propuestas en la International Collection of Arbuscular Mycorrhizal Fungi y la AMF phylogeny. Del primer muestreo se identificaron 11 especies incluidas en siete géneros: *Glomus* con dos especies no determinadas, *Glomus* sp.1, *Glomus* sp.2, *Gl. etunicatum* y *Gl. geosporum*; género *Acaulospora*, una especie no detrminada *Acaulospora* sp., *A. spinosa*, *A. birreticulata* y *A. denticulata*; del género *Entrophospora*, *E. infrecuens*; del género *Diversispora*, *D. aurantia;* del género *Scutellospora*, *S. pellucida;* del género *Racocetra*, *R. castanea* y *R. verrucosa* y del género *Gigaspora*; *Gi. decipiens*. En el segundo y tercer muestreo, no se observó la presencia de nuevos géneros de HMA pero si aumentó la cantidad de esporas (en promedio 38.09% y 30% respectivamente). La aplicación de estas especies del género *Trichoderma* para el control de fitopatógenos de la raíz de aguacate favoreció el incremento de esporas HMA en la rizosfera de este cultivo.

Palabras clave: *Phytophthora cinnamomi*; *Trichoderma*; aguacate; control biológico.

INTRODUCTION

Agro-ecological conditions where the avocados are cultivated in the state of Michoacan, Mexico, have led to pests and fungal diseases of various kinds and are closely associated with the commercial development of the crop, including the avocado´s sadness caused by the Oomycete *Phytophthora cinnamomi* Rands causing great economic losses for producers (Sánchez, 2007). This disease has been for avocado producers, the main limiting factor to economic production in countries such as Australia, Mexico, South Africa and the United States (Pegg *et al*., 2002).

The use of microorganisms is an important agricultural practice, their use as biofertilizers can help provide nutrients, phytohormones and antagonist as biological control agent. The main microorganisms in soil capable of achieving these effects are species of the genus *Trichoderma* and arbuscular mycorrhizal fungi (AMF) (Parets, 2002; Bolaños and Sáenz, 2009). It has been shown to interact in the biological control of plant pathogens in the soil and improve the development of mycorrhizal symbionts; this interaction has effects on the growth and health of the host plant (Calvet *et al.*, 1993; Godeas *et al*., 1999).

Arbuscular mycorrhizal symbiosis in avocado plants in the last 25 years has been important because they improve their growth and the absorption of soil nutrients, particularly phosphorus (Peterson *et al*., 1984). Ginsburg and Avizohar-Hershenson (1965) were the first to determine the mycorrhizal association in roots at avocado orchards in Israel, while in California Menge *et al.* (1978) noted the benefits of AMF on growth of avocado and reducing damage caused by transplantation. Later Menge *et al*. (1980) found that the presence of mycorrhiza at plants of avocado "Topa Topa" increased absorption of the nutrients N, P, Cu and Zn, compared to plants without mycorrhiza. In similar studies with avocado plants was observed that micro-propagated plants further growth and uptake of N, P and K, as well as improved acclimatization once they were transplanted. These studies indicate that the roots colonized by mycorrhizal fungi promote plant growth and improve the absorption of nutrients and their health (Vidal *et al*., 1992; Lahav and Whilen, 2007).

The populations of beneficial microorganisms found are shaped in the ground potential, they are antagonistic to important plant pathogens as *Phytophthora cinnamomi* (Reyes *et al.*, 1997). Arbuscular mycorrhizal fungi are sometimes up to 50% of the biomass of soil microorganisms (Xavier and Germida, 1999) they increase the absorption of water and nutrients, host plant has more vigorous growth and greater resistance to adverse environmental conditions caused by water stress, pH, temperature extremes and high concentrations of heavy metals (Harley and Smith, 1983). Mycorrhizal colonization can also protect the plant roots of certain pathogens and improving water relations; in addition, extraradical hyphae can contribute significantly to soil aggregation and structural stability (Ryan and Gram, 2002).

Avocado root has no root hairs, it is likely that this lack is due to the abundance of moisture and organic matter in tropical forests that evolved this crop (Salazar, 2002), mycorrhiza is present in natural habitats where the avocado is native, although the presence of the symbiosis in the field and the role they may have within the production system has not been determined with certainty; however, have been successful *in vitro* propagation and nursery (Reyes *et al.,* 1997) Because of this, the use of arbuscular

mycorrhizal fungi can help the speedy recovery of trees affected by *P. cinnamomi*, also this practice would help to reduce pesticide use, promotes soil microbial activity and would increase the resistance of avocado plants to this and other soil pathogens (Lara, 1998).

For its part, the genus *Trichoderma* has good qualities to control plant diseases caused by phytopathogenic soil fungi, mainly of the genus *Phytophthora, Rhizoctonia, Sclerotium*, *Pythium* and *Fusarium*, without affecting the beneficial microorganisms, acting as competitive hyperparasites produce antifungal metabolites and hydrolytic enzymes to which they attribute the structural changes at the cellular level, such as vacuolization, granulation, disintegration of the cytoplasm and cell lysis (Papavizas *et al*., 1982). The mechanisms for the displacement of plant pathogens induced by *Trichoderma* are of three types: direct competition for space and nutrients (Elad and Baker 1985, Elad and Chet 1987, Chet and Ibar 1994, Belanger *et al*., 1995), production of antibiotic metabolites volatile or nonvolatile (Chet *et al*., 1997; Sid Ahmed *et al*., 2000; Sid-Ahmed *et al*., 2003) and direct parasitism on fungal pathogens (Yedidia *et al.*, 1999; Ezziyyani *et al*., 2003). The objective of this study was to determine the presence of arbuscular mycorrhizal fungi (AMF) in the rhizosphere of avocado trees affected by *Phytophthora cinnamomi* under biological control.

MATERIAL AND METHODS

Research site description

The study was conducted from august 2009 to september 2010 in the avocado orchard called "Eye Water" in the town of Tancítaro, Michoacan, Mexico (19 ° 20 'N and 102 ° 22' W, 2080 masl), owned by Mr. Jesús Alejandro Guerrero Tejeda, their avocado trees are infected with *Phytophthora cinnamomi*.

The climate of the region is A W1 (W) corresponding to the warm humid with rains in summer, winter rain and less than 5% intermediate moisture (Garcia, 1973), with rainfall from 800 to 1500 mm per year and a temperature of 10 to 26 °C. The relative humidity is 90 % predominant (Lara, 2005). The soil is classified as Andosol (Lara, 2005; EDISA, 2003).

Sampling

21 avocado trees 25 years old infected with *P. cinnamomi* were selected, soil samples were taken (1 kg) from rhizosphere area in the first 30 cm depth of soil (Bagyaraj, 1991), were performed three sampling periods: august 9, 2009, february 9 and september 21, 2010. The trees showed level III affectation in accord with Coffey's scale modified by Lara (2008). For the control of *P. cinnamomi* were applied three strains endemics in the avocado Michoacan's region of *Trichoderma* (*Trichoderma erinaceum* Bissett, Kubicek & Szackacs, *Trichoderma aggressivum* Samuels & W. Gams and *Trichoderma arundinaceum* Zafari, Graef. & Samuels from ceparium of Laborario de Fitopatología de la Facultad de Agrobiología, Universidad Michoacana de San Nicolás de Hidalgo), they were spray to the root as conidia solution $1{,}92\times10^4$ conidia/mL, before its application, carried out soil sampling to determine AMF spores.

Extraction and identification of AMF species

Samples were processed in the laboratory of plant pathology at the Facultad de Agrobiología "Presidente Juarez" UMSNH, which was conducted to determine the species of arbuscular mycorrhizal fungi in rhizosphere of avocado trees infected by *P. cinnamomi*, before and after of the treatments with *Tricoderma* species. To recover the soil AMF spores was used wet sieving and decantation protocol proposed by Gerdemann and Nicolson (1963), followed by centrifugation on sucrose (400 g L-1) at 2000 rpm. Taxonomic identification was made under a compound microscope (Leica DM 1000), morphological characteristics were recorded as the shape, size and color of the spores, thickness, ornamentations, number of layers of the wall and the coupling form and supporting hyphae, identifications were made by comparison with original descriptions and nomenclature of Shübler and Walker (2010). To perform the counting of the spores were placed in Petri dishes, to which was placed a grid of 1.0 cm X 1.0 cm was cut in a circle similar to the bottom of the Petri dish, the total number of spores was obtained of a subsample of 100 g of soil and observed under a stereoscopic microscope (Leica) (Ferrera *et al*., 2005; INVAM, 2010).

RESULTS AND DISCUSSION

In this research, 14 different AMF morphotypes were recorded in avocado trees infected with *P. cinnamomi.* The AMF are distributed into two orders: Glomerales y Diversisporales, four families: Acaulosporaceae, Entrophosporaceae, Gigasporaceae y Glomeraceae, and seven genera: *Acaulospora*, *Diversispora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Racocetra* and *Scutellospora*. We found only fungal species representatives of the orders Glomerales and

Diversisporales, but not of Paraglomerales and Archaeosporales.

The Order of Glomerales: were identified three species and two undetermined species in the family Glomeraceae, Genus *Glomus*, *Glomus* sp.1, *Glomus* sp.2, *Glomus aurantium* (Blaszk. V. Blanke, C. Renker and Buscot F.), *Glomus etunicatum* (Becker and Gerdemann) and *Glomus geosporum* (Nicolson and Gerdemann) Walker, this family was the best represented by the number of morphospecies registered.

The Order Diversisporales with three families Acaulosporaceae, Diversisporaceae and Gigasporaceae; from the family Acaulosporaceae with the genus *Acaulospora* with three species and one undetermined species; *Acaulospora* sp., *Acaulospora spinosa* Walker & Trappe, *Acaulospora bireticulata* (Rthweell & Trappe) and *Acaulospora denticulata* (Sieverding & Toro). From Diversisporaceae family, genus *Diversispora* with one species *Diversispora aurantia* (Braszk, Blanke, Render & Buscot). From Gigasporaceae family, *Racocetra*, *Scutellospora* and *Gigaspora* genera, of the genus *Racocetra*, two species *Racocetra castanea* (C. Walker) and Racocetra *verrucosa* (Koske & Walker), of *Scutellospora* one species, *Scutellospora pellucida* (Nicolson & Schenck) and the genus *Gigaspora*, *Gigaspora decipiens* Hall & Abbott. Finally, *Entrophosporaceae* family with a molecular phylogenetic and systematic position unknown (Schübler y Walker, 2010), recorded the *Entrophospora* genus, species *Entrophospora infrequens* (I. R. Hall).

The relative abundance of species in response to the application of control treatments on *P. cinnamomi* was different, when the three species of *Trichoderma* were used, in the second sampling, six months after the first application, despite having met representatives of the five genera already described, the number of fungal spores in these increased by 38.09% on average compared with the first sampling where *Trichoderma* was not applied. In the third sampling was lowering compared to the second (30%). It is noteworthy that the second sampling was in the dry season and the orchard is temporary. Almaraz and Reyes (2007) observed an increase in the number of spores and species associated to variation had avocado orchards irrigation and temporary, during the rainy season. Gonzalez (2005) indicates that the number of spores in a temperate forest of pine-oak is more stable in dry and rainy seasons, while in an avocado orchard in the rainy season significantly increases the number of spores. Moreover, Larsen and Ranskov (2004) indicate that when using *Trichoderma* strains and AM fungal strains to control plant pathogens in roots of cucumber, leek and tomato, observed an increase in the number of mycorrhizal spores but not in the fungal kind, this due to greater number of healthy roots of plants, indicating that AMF are compatible with other biological control agents and help the health of plants.

CONCLUSIONS

We registered 14 morphospecies of arbuscular mycorrhizal fungi included in seven genera*: Acaulospora*, *Diversispora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Racocetra* and *Scutellospora*. The genera that had the highest number of species were *Glomus* and *Acaulospora*, while those with the fewest were *Entrophospora* and *Gigaspora*. Although avocado trees affected by *Phytophthora cinnamomi* have a reduced root system, it is possible to find AMF spores and an important AMF diversity in the rhizosphere of the avocado trees.

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