ARBUSCULAR MYCORRHIZAL COLONIZATION IN AVOCADO ORCHARDS WITH TWO DIFFERENT FARM MANAGEMENT PRACTICES

Abigail Balderas-Alba, Gregorio Luna-Esquivel and Rocío Vega-Frutis

SUMMARY

Mexico is the largest avocado producer worldwide. However, this condition has increased the use of agrochemicals and, thus, has reduced the biotic below-ground interactions, such as those between plants and arbuscular mycorrhizal (AM) fungi. AM fungi can contribute to the restoration of degraded ecosystems. Therefore, we quantified the infectivity by these fungi in two avocado orchards with different farm nutrient management practices, in the state of Nayarit, Mexico. In agreement with other studies, our results showed that chemical fertilizers had negative effects on the intraradical fungal structures, and the orchard that received agrochemicals had a lower mycorrhizal colonization. The implications that agrochemicals have on arbuscular mycorrhizal symbiosis are discussed.

Introduction

Anthropogenic activities such as agricultural intensification, deforestation, urban expansion and industrial activities are some of the main global causes of above- and below-ground biodiversity decline in terrestrial ecosystems (Newbold *et al*., 2015). The conversion of natural vegetation into agricultural fields is known to change the structure and functional traits of soil microorganisms (Lambin *et al*., 2000; Morris and Blackwood, 2015; Voroney and Heck, 2015), as well as to negatively affect particle aggregation, nutrient leaching, nutrient cycling, and below-ground biotic interactions (Wagg *et al*., 2014).

One of the most important components in the majority of terrestrial ecosystems is the mutualistic association between plants and arbuscular mycorrhizal (AM) fungi belonging to the phylum Glomeromycota (Smith and Read, 2008). AM fungi play an important role in providing benefits and services to agroecosystems, such as increasing soil adherence, soil stability and water retention, and thus, promoting soil health and plant quality (Gianinazzi *et al*., 2010). AM fungi transfer water and mineral nutrients such as phosphorus, nitrogen, and micronutrients to their host plants through the hyphae that grow into the soil matrix. The mineral nutrients are translocated inside the roots through symbiotic-specific structures and, in exchange, the fungi receive carbohydrates for their survival and growth (Smith and Read, 2008). In consequence, mycorrhizal associations can act as a buffer of biotic (Gehring and Bennett, 2009) and abiotic (Arafat *et al*., 2016) stresses. There is evidence that the infectivity (ability of fungal propagules to colonize host plant and deliver the mineral nutrients into the roots) and effectiveness (mycorrhizal plant benefit in terms of growth and fitness compared with non-mycorrhizal plants), along with AM richness decreases as land use intensifies (Moora *et al*., 2014; Wagg *et al*., 2014; Trejo *et al*., 2016; Vega-Frutis *et al*., 2018).

Mexico is the avocado (*Persea americana*) center of origin (Smith *et al*., 1966) and the state of Nayarit is the fourth national producer with a total of 49,245.79 tons produced in 2017 (SIAP, 2018). Some studies have reported that the avocado is a mycotrophic tree, and most studies have evaluated the plant performance using commercial and native mycorrhizal inocula (Vidal *et al*., 1992; Carreón-Abud *et al*., 2015; Castro *et al*., 2013). However, little attention has been paid to how farm management practices affect the infectivity and effectivity of AM fungi. In the present study, we quantified the AM colonization from two avocado orchards with different farm nutrient management practices. Because, in general, agrochemicals have a negative effect on AM colonization, we predicted lower root colonization by AM fungi in trees from the orchard that receives chemical fertilizers.

Materials and Methods

Study site

This study was performed in the municipality of Xalisco, Nayarit, Mexico, in two avocado (*Persea americana*, Hass variety) orchards with different farm nutrient management practices. The location of each orchard is presented in Table I. Orchard 1 did not receive chemical nutrients, while orchard 2 received a combination

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COLONIZACIÓN MICORRÍZICA ARBUSCULAR EN PARCELAS DE AGUACATE CON DIFERENTES PRÁCTICAS DE MANEJO AGRÍCOLA

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RESUMEN

México es el mayor productor de aguacate en el mundo. Sin embargo, ello ha incrementado el uso de agroquímicos y, por lo tanto, han disminuido las interacciones bióticas del suelo, como es el caso de la micorriza arbuscular. Los hongos micorrizógenos arbusculares pueden contribuir a la restauración de los ecosistemas degradados. Por lo tanto, cuantificamos la infectividad por estos hongos en dos parcelas de aguacate con diferentes prácticas de manejo de nutrientes, en el estado de Nayarit, México. En concordancia con otros estudios, los resultados mostraron que el fertilizante químico tuvo efectos negativos en las estructuras fúngicas intrarradicales, y la parcela que recibe agroquímicos tuvo la menor colonización micorrízica. Se discuten las implicaciones que los agroquímicos tienen sobre la simbiosis micorrízica arbuscular.

COLONIZAÇÃO MICORRÍZICA ARBUSCULAR EM PARCELAS DE ABACATE COM DIFERENTES PRÁTICAS DE MANEJO AGRÍCOLA

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RESUMO

O México é o maior produtor de abacate no mundo. No entanto, isso tem aumentado o uso de agroquímicos e, por conseguinte, as interações bióticas do solo como as micorrizas arbusculares, tem diminuído. Os fungos micorrízicos arbusculares podem contribuir na restauração de ecossistemas degradados. Portanto, quantificamos a infectividade desses fungos em duas parcelas de abacate com diferentes práticas de manejo de nutrientes, no estado de Nayarit, México. Em conformidade com outros estudos, os resultados mostraram que o fertilizante químico teve efeitos negativos nas estruturas fúngicasintra-radicais, e a parcela que recebe agroquímicos apresentou a menor colonização micorrízica. Discutimos as implicações que os agroquímicos têm na simbiose micorrízicaarbuscular.

of nutrients, both chemical (N, P, K, Ca, Zn, B, and Mg, ~4kg/tree per year) and organic (chicken manure, 30kg/tree per year).

Arbuscular mycorrhizal colonization measurements

In each orchard, fine roots from 30 randomly selected reproductive avocado trees were collected in two plots of 50×50m (15 trees per plot), and recorded their basal stem circumference (BSC) on September 2017. In agreement with Vega-Frutis *et al*. (2015), the roots were collected from a radius of 1 to 1.5m around the tree trunk. The roots were placed in ethanol (50% v/v), processed according to the method of Koske and Gemma (1989) and stained with trypan blue (0.05%). We calculated the colonization percentage by fungal structures (hyphae, vesicles and arbuscules) in 15 root fragments (each ~1.5cm long) from each tree. Each root fragment was examined at three equally spaced points under a light microscope at 100× and 400× total magnifications, using the cross-hair intersection method (McGonigle *et al*., 1990).

Soil chemistry

Five samples of soil per plot (10 samples per orchard) were collected. The soil was homogenized and a subsample of ~1kg per orchard taken for chemical analyses (Table I). Chemical soil parameters were determined according to the Mexican official standard (SEMARNAT, 2002) and were carried out at the Laboratory of Soil Analyses of the Ecology Institute A.C., in Xalapa, Mexico.

Data analysis

A nested ANOVA model was used to explore differences in the basal stem circumference (BSC) between the trees of each orchard. The plot was nested within orchard. To meet the model assumptions (normality of residuals and the homogeny of variances), the BSC was transformed to Neperian logarithms. With test for differences in the percentage of colonization by hyphae, vesicles and arbuscules, we used nested ANCOVA models (the plot was nested within orchard). Given that there were significant differences in the BSC between the orchards, the BSC was used as a covariable. To meet the model assumptions (normality of residuals and the homogeny of variances), the percentages were arcsine transformed. The data analyses were conducted with the statistical language R (R Core Team, 2016).

Results and Discussion

There were significant differences in the basal stem circumference ($F_{1,56}$= 25.44, P<0.001). The trees in orchard 2 had a higher BSC (mean ±standard error, 103.43 ±4.15cm), compared with orchard 1 (73.88±4.93cm). Opposite, the percentage of colonization by hyphae, vesicles and arbuscules were higher in orchard 1 compared to orchard 2 (Table II), and these differences were statistically significant (Table III). Although the trees showed differences in their BSC between orchards, the BSC did not affect the fungal intraradical structures (Table III).

The study showed that trees growing in orchard 2 had the lowest AM colonization for all quantified fungal structures. Also, this orchard had, overall, the biggest trees. These results agree with our initial prediction. The findings concur with several studies showing that agrochemicals have a negative effect not only on mycorrhizal colonization but also on fungal propagules (Wagg *et al*., 2014;

TABLE I
LOCALIZATION AND SOIL FERTILITY PARAMETERS FROM TWO AVOCADO ORCHARDS IN NAYARIT, MEXICO

	Orchard	
	1	2
Coordinates	21°24'05.1"N, 104°56'56.2"W	21°23'55.1"N, 104°56'18.3"W
Soil density	2.84	2.40
Apparent density	0.72	0.76
Ca	7.24	4.95
Mg	5.20	4.33
K	1.05	1.18
CEC	13.32	12.88
Fe	106.00	62.90
Mn	7.40	3.90
Cu	0.30	3.10
Zn	2.10	1.00
pH	5.88	5.58
P	51.00	7.00
OM	5.44	4.09
OC	3.16	2.37
TC	5.73	3.48
TH	0.39	0.84
TN	0.64	0.61
C/N	9.00	6.00
Clay	28.20	32.20
Silt	20.00	26.00
Sand	51.80	41.80
Texture	Sandy clay loam	Clay loam

Soil density and apparent density: g/cm^3. Calcium (Ca), magnesium (Mg), potassium (K) and cation exchange capacity (CEC): acetate NH_4pH 7 (cmol/kg). Iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn): DTPA (mg/kg). pH: 1:2 H_2O. Phosphorus (P) Bray Kurtz: mg/kg. OM: organic matter, OC: organic carbon, TC: total carbon, TH: total hydrogen (TH) and TN: total nitrogen: %. C/N: carbon/nitrogen. Clay, silt and sand: %.

TABLE II
FUNGAL AM PARAMETERS ANALYZED IN *Persea americana* TREES

Orchard	Hyphae (%)	Vesicles (%)	Arbuscules (%)
1	82.25 ± 4.30a	76.76 ± 4.60a	46.04 ± 4.10a
2	63.01 ± 4.75b	60.88 ± 4.89b	23.19 ± 3.51b

Means ±standard errors. Different letters indicate statistically significant differences between orchards.

TABLE III
SUMMARY OF STATISTICAL RESULTS OF NESTED ANCOVA MODELS FOR FUNGAL STRUCTURES ANALYZED IN AVOCADO TREES

Source of variation	df	Hyphae (%)		Vesicles (%)		Arbuscules (%)	
		F	P	F	P	F	P
Orchard	1	16.61	<0.001	9.10	0.004	26.17	<0.001
BSC	1	0.88	0.352	0.95	0.333	0.45	0.505
Orchard:BSC	1	3.03	0.087	2.92	0.093	1.84	0.18
Orchard:Plot	2	7.79	0.001	7.38	0.001	13.79	<0.001
Residuals	54						

BSC: basal stem circumference.

Trejo *et al*., 2016; Vega-Frutis *et al*., 2018). For instance, Vega-Frutis *et al*. (2018) found that the frequency of AM colonization in wild papaya decreased as the disturbance increased. The same pattern was observed by Oehl *et al*. (2003), who observed that the frequency of AM colonization was lower in the case of monocropping. In the same way, the richness of AM fungi was lower in spruce plantations, pasture or monocropping, compared with other ecosystems such as forests or grasslands (Moora *et al*., 2014; Trejo *et al*., 2016).

Although the avocado trees in both orchards were colonized by AM fungi, including arbuscules (specialized structures for nutrient exchange between fungi and their host plants), orchard 2, as mentioned above, had the lowest percentage of AM fungi. Likely, the intraradical fungal structures observed in the orchard 2 were due to the large number of herbaceous species that cover the soil between avocado trees, which provide many potential host plants. Therefore, fungal propagules could benefit the avocado trees in terms of growth, reproduction and tolerance to pathogens and herbivores (Lara-Chávez *et al*., 2013). In addition, this orchard receives a combination of chemical and organic nutrients. The nutrient enrichment reduces the allocation of resources to the root and AM fungi, and the resources could be allocated to the aerial structures (Johnson, 2010). This could explain the greater size (basal stem circumference) of the trees in orchard 2, i.e., the use of agrochemicals has a positive effect on yield, but negative effects on below-ground microorganisms (Gagic *et al*., 2017).

Several studies have shown that increasing soil P availability significantly reduces the P uptake pathway in mycorrhizal fungi (Konvalinková *et al*., 2017). In this study, orchard 1 had the highest AM colonization, but also a higher total soil P. We did not quantify the soil P available and, therefore, it is probable that the amount of P available for avocado trees might be lower than in orchard 2. This could explain the high AM colonization in orchard 1. In addition, it was observed that avocado plants growing with soil from orchard 1 had greater root diameter, lower specific root length and root branching ratio (unpublished data), suggesting a high mycorrhizal dependency in avocado trees growing in soils with lower available mineral nutrients (Vega-Frutis *et al*., 2015; Wen *et al*., 2019).

Some studies have suggested that avocado plants have a mycorrhizal growth ‘dependence’. For instance, Gómez *et al*. (2012) observed percentages of mycorrhizal colonization of ~80 to 100%. However, plant species show intraspecific variation in root morphology and in the degree of AM colonization in response to soil P (Wen *et al*., 2019). Therefore, integrative studies are needed on the relationship between crop management practices and the plasticity in root morphology with the availability of soil P to drive sustainable agricultural practices and improve ecosystem services (Rilling *et al*., 2019).

Conclusions

To our knowledge, no studies have been performed on economical plants and their AM fungi in the state of Nayarit. Our study shows that management practices decrease AM colonization. The AM fungi can reduce the use of agrochemicals, promoting soil health, plant performance and quality in agroecosystems. Therefore, more research on the interactive effects of management practices and mycorrhizal symbiosis in avocado crops is needed; given that no attention has been paid to this topic in the state of Nayarit despite it being the fourth largest producer of avocado in Mexico.

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