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“Infectividad y efectividad de hongos micorrícicos arbusculares en aguacate (*Persea americana*), Xalisco; Nayarit”

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Con base al oficio de fecha 03 de octubre del presente, enviado por los **CC. Dra. María del Rocío Vega Frutis, Dra. Gabriela Rosario Peña Sandoval, y Dr. Gregorio Luna Esquivel,** donde se indica que el trabajo de tesis cumple con lo establecido en forma y contenido, y debido a que ha finalizado con los demás requisitos que establece nuestra institución, se autoriza a la **IBQ. Itzel Abigail Balderas Alba,** continúe con los trámites necesarios para la presentación del examen de grado de Maestría en Ciencias Biológico Agropecuarias en el Área de Ciencias Ambientales.

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Los suscritos integrantes del Cuerpo Tutorial para asesorar la Tesis titulada: Infectividad y efectividad de hongos micorrícicos arbusculares en aguacate (*Persea americana* L.), Xalisco; Nayarit, que presenta la **C. Itzel Abigail Balderas Alba** para obtener el Grado de Maestra en Ciencias con opción terminal en Ciencias Ambientales, damos nuestra aprobación para que continúe con los trámites correspondientes para la obtención de su grado.

Sin otro asunto que tratar, reciba un cordial saludo.

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A los seres que amo

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CAPÍTULO I

INTRODUCCIÓN GENERAL

La sociedad ha reconocido su dependencia del suelo, y se ha planteado que contienen un componente biológicamente activo, que es importante junto con su diversidad en la estabilidad y función de los procesos de los ecosistemas (Wardle, 2002). Entender los procesos del suelo, es vital para alcanzar una productividad agrícola sustentable, así como conservar la biodiversidad, restaurar y/o rehabilitar los ecosistemas perturbados (Álvarez-Sánchez, 2009).

Los hongos micorrizógenos arbusculares (HMA, Subphylum Glomeromycotina) son un grupo clave de la biota del suelo, son simbiontes obligados que colonizan las raíces de la mayoría de las especies de plantas terrestres (briofitas, pteridofitas, gimnospermas y angiospermas), y se encuentran en casi todos los ecosistemas terrestres (Smith y Read, 2008; Spatafora et al., 2016).

La simbiosis micorrícica implica un intercambio bidireccional de nutrientes, donde las plantas reciben principalmente fósforo y agua, y en respuesta, los hongos obtienen de su planta hospedera carbohidratos esenciales para su crecimiento y reproducción. Se ha estimado que aproximadamente el 30% de los carbohidratos producidos durante la fotosíntesis pasan al hongo (Jakobsen y Rosendhal, 1990; Drigo et al., 2010). A cambio, los hongos, forman una extensa red de hifas a través de las cuales absorben nutrientes minerales (p. ej. fósforo y nitrógeno) más eficientemente que las raíces y transportan estos minerales a estructuras fúngicas que se forman dentro de las raíces (Smith y Read, 2008, Figura 1). Dentro de las raíces, las hifas forman los arbúsculos y los ovillos, que son las principales estructuras para el intercambio de nutrientes entre los simbiontes, además de vesículas que son las estructuras de reserva (principalmente de lípidos) del hongo, y algunas especies de hongos producen esporas (Klironomos y Hart, 2002; Requena et al., 2007; Smith y Read, 2008, Figura 1). Se ha observado que las plantas, además de mejorar su estatus nutricional, son más resistentes al estrés biótico (p. ej. patógenos y herbívoros,

Gehring y Bennett, 2009) y abiótico (p. ej. salinidad, sequía, metales pesados, Miransari, 2010; Arafat et al., 2016).

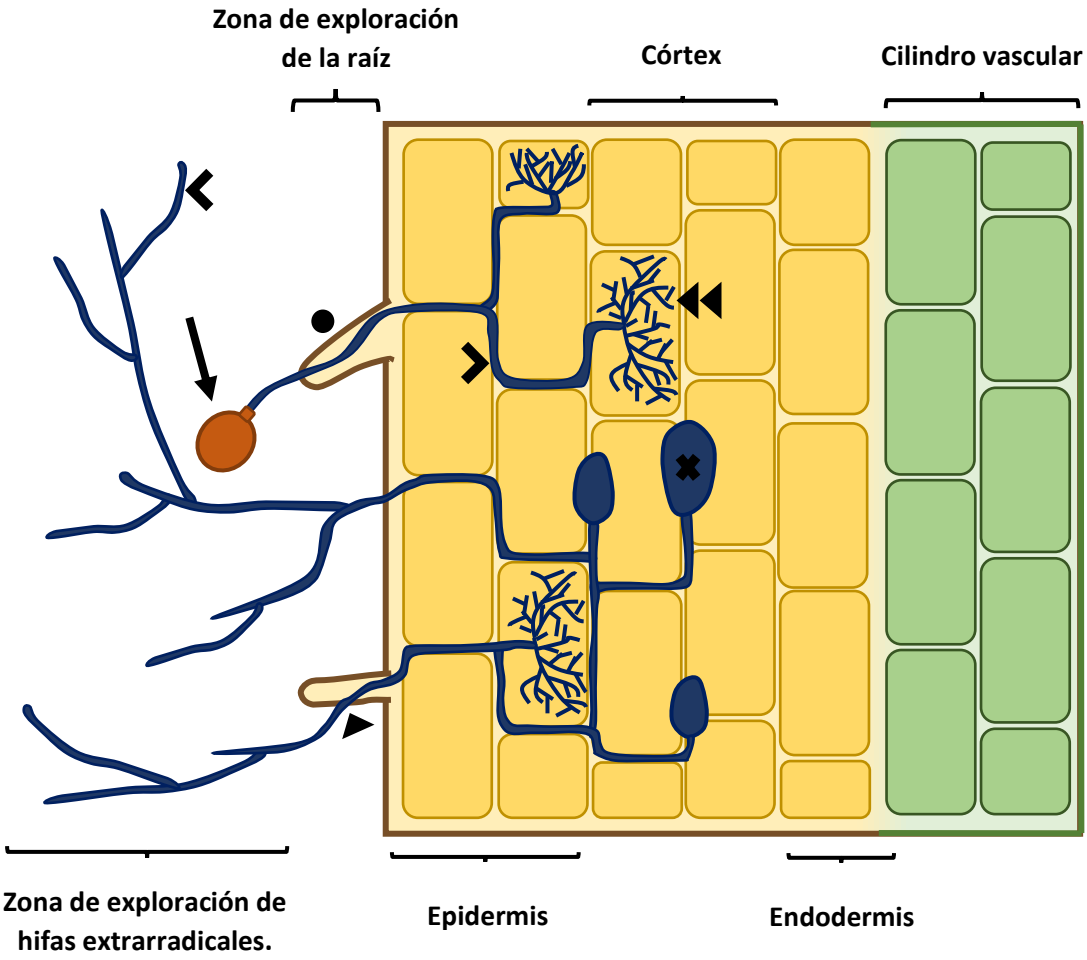

Figura 1 Representación gráfica de las principales estructuras fúngicas de las micorrizas arbusculares, fuera y dentro de la raíz de la planta. Los HMA, fuera de la raíz tienen la capacidad de formar **(→)** esporas, las cuales son estructuras de propagación, latencia y la única fase independiente del hongo. El **(►)** apresorio, es el sitio de penetración de la **(<)** hifa extrarradical para establecer la colonización y a su vez, absorber fósforo, nitrógeno, micronutrientes y agua de manera más eficiente que los **(●)** pelos radiculares. Dentro de la raíz, se forman los **(◄◄)** arbúsculos, los cuales son el principal sitio de intercambio de nutrientes, **(>)** hifas intrarradicales y las **(X)** vesículas, que son estructuras de reserva del hongo, almacenando principalmente lípidos. Estas estructuras fúngicas se forman en la epidermis y/o córtex de la planta, sin invadir la endodermis y el cilindro vascular.

Los HMA también tienen un papel en la estabilidad y calidad del suelo, en la capacidad de retención de agua y la redistribución de minerales, son esenciales para la rehabilitación de suelos, y para la sustentabilidad de los (agro)ecosistemas (Klironomos et al., 2000; Álvarez-Sánchez, 2009).

Existen pocos estudios sobre la diversidad y complementariedad funcional de estos hongos en los diferentes (agro)ecosistemas. Se ha documentado que, aunque los HMA no son estrictamente hospedero-específicos, si exhiben un grado de preferencia por algunas plantas hospederas, además de que las plantas están colonizadas por varias especies de hongos (Öpik et al., 2009). Aunque se ha demostrado que muchas especies de HMA se pueden encontrar ampliamente distribuidas en diversos tipos de vegetación semejantes o contrastantes, existen también especies asociadas solamente a un tipo de ecosistema (Öpik et al., 2006, 2010). Por lo tanto, es prioridad conocer las especies de HMA que se encuentran en cada tipo de (agro)ecosistemas con la finalidad de conocer las especies nativas y su papel, no solo en el desempeño de las plantas, también en el ecosistema y su uso como bioinoculantes (Lauriano-Barajas y Vega-Frutis, 2018).

Se ha documentado que los monocultivos, la intensificación de la agricultura, así como la aplicación de plaguicidas y la elevada adición de nutrientes disminuyen la presencia de los HMA (An et al., 1993; Oehl et al., 2003; Hijri et al., 2006; Thompson et al., 2009; Oehl et al., 2010). Además, la fertilización desmedida, puede potencialmente generar una relación competitiva entre las plantas y los HMA, y causar depresión en el crecimiento de las plantas, así como reducir la biomasa fúngica (Johnson, 2010, Figura 2). Por otra parte, la labranza y otras formas de alteración del suelo rompen las redes de hifas de los HMA presentes en su hábitat, lo que afecta la capacidad de las micorrizas para colonizar las raíces de las plantas, y reduce la absorción y el transporte de nutrientes hacia las plantas hospederas (Kabir, 2005). Además de disminuir los propágulos fúngicos y su viabilidad a largo plazo (Trejo et al., 2016).

Para el estado de Nayarit no existen estudios sobre la infectividad y efectividad de las micorrizas arbusculares en cultivos de importancia económica (Montaño et al., 2012). Sin embargo, recientemente se ha documentado que el cambio de uso de suelo afecta la colonización intrarradical (hifas, vesículas y arbúsculos) en plantas de papaya silvestre, distribuidas en tres sitios de Nayarit con diferente intensidad de perturbación (Vega-Frutis et al., 2018).

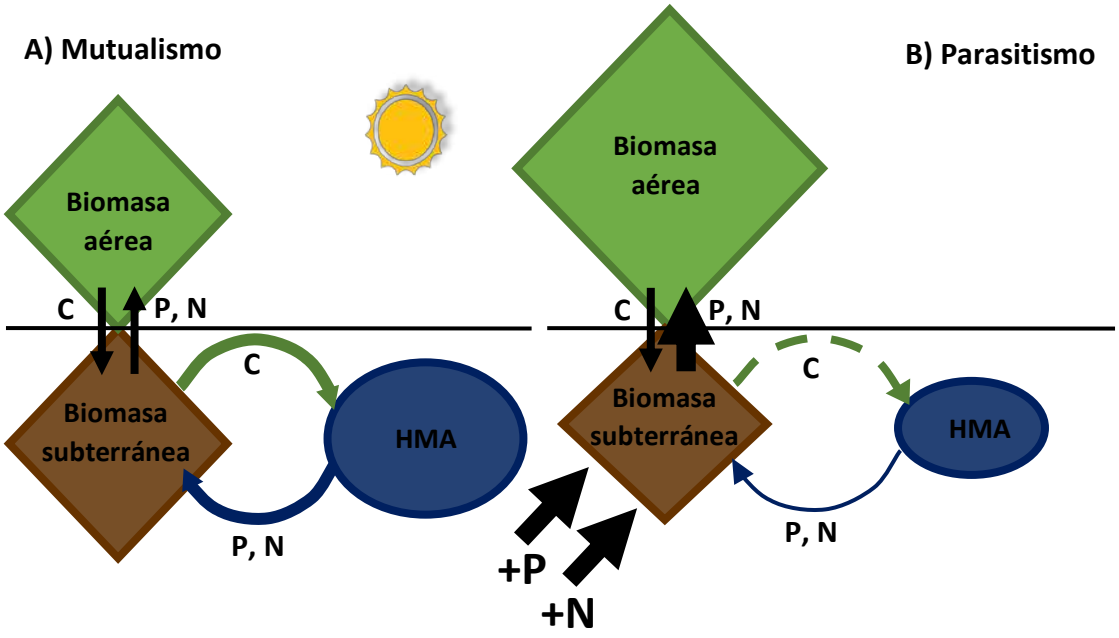

Figura 2 Interacción de las plantas con los hongos micorrizógenos arbusculares de acuerdo a la disponibilidad de recursos. A) Mutualismo, existe un balance entre la planta y el hongo. La planta proporciona carbono (C) y recibe fósforo (P) y nitrógeno (N) por parte de los HMA. Flechas del mismo grosor representan el equilibrio entre los flujos de los elementos. B) Parasitismo, con el enriquecimiento de N y P, las plantas reducen la asignación de recursos a las raíces y los hongos, y asignan recursos a las estructuras aéreas. Flechas con diverso grosor y discontinuas representan el desequilibrio entre los flujos de los elementos (Esquema modificado de Johnson, 2010).

ANTECEDENTES

Cambio de uso de suelo

El suelo es un ambiente muy complejo, se ha estimado que contiene aproximadamente un tercio de todos los organismos vivos y regula la actividad de los organismos responsables del funcionamiento y evolución de los ecosistemas (Voroney y Heck, 2015). El suelo proporciona servicios ecosistémicos esenciales, que están cambiando cuantitativa y cualitativamente de acuerdo con las condiciones medioambientales, las cuales responden a ciclos abióticos (p. ej. temperatura, humedad), además de los organismos del suelo y las plantas.

Los factores bióticos y abióticos juegan un papel importante en la formación y funcionamiento de los suelos (Voroney y Heck, 2015). Sin embargo, las prácticas de manejo forestal y agrícola, incluyendo la fragmentación del hábitat para cultivos, carreteras, vivienda, etcétera (Verburg et al., 2015) causan efectos deletéreos a los organismos del suelo y sus propiedades.

De acuerdo con Ellis et al. (2010), para el año 2000, aproximadamente el 55% de los ecosistemas terrestres habían sido convertidos en pastos, tierras de cultivo, asentamientos y otros usos de suelo. El deterioro ambiental ha llevado a la extinción de un gran número de especies (era del antropoceno), comparándose con las cinco grandes extinciones de la Tierra (Dirzo et al., 2014). Esto ha tenido un impacto en el clima, en la introducción de especies exóticas y en la pérdida de hábitats, afectando la diversidad de plantas, animales y sus interacciones bióticas. Por ejemplo, se ha modificado la distribución, fenología, abundancia, y fisiología de las plantas y sus polinizadores, tanto en comunidades naturales como agrícolas (Salazar et al., 2016).

La intensificación de la agricultura afecta la diversidad de bacterias, actinomicetos, protozoarios y hongos en el suelo (Helgason et al., 1998; Mäder et al., 2002; de Vries et al., 2013). Esto ha generado una creciente preocupación dado que la biodiversidad del suelo juega un papel importante en la descomposición de la

materia orgánica, el ciclo de nutrientes, la bioturbación, la retención de agua, y la estabilidad del suelo (Helgason et al., 1998; van Der Heijden et al., 2008; Wall et al., 2010; de Vries et al., 2013).

En México existe una elevada diversidad biológica y cultural, pero un profundo deterioro de los ecosistemas que albergan esta biodiversidad (Balvanera y Cotler, 2009). Por lo tanto, nuevas alternativas han surgido para contrarrestar el impacto causado por el cambio de uso de suelo, y disminuir el uso de agroquímicos, las cuales consisten en la aplicación de microorganismos a las semillas, superficie de las plantas o al suelo (Vessey, 2003; Malusá et al., 2012; Pavithra y Yapa, 2018).

Recientemente, los hongos micorrizógenos arbusculares (HMA) se han considerado como una alternativa para disminuir el uso de agroquímicos, debido a que mejoran el estatus nutricional de las plantas, en consecuencia, disminuye la aplicación de agroquímicos. Sin embargo, existen diferencias en los resultados de estos efectos de acuerdo con las distintas especies de plantas y HMA involucrados (Cuenca, 2015).

Cambio de uso de suelo y simbiosis micorrícica

Debido a su ubicuidad, los HMA proveen varios servicios ecosistémicos (Öpik et al., 2006). Sin embargo, se sabe que los propágulos fúngicos (esporas, fragmentos de raíces colonizadas y micelio extrarradical, Figura 1) se ven afectados por la perturbación y el cambio en el uso del suelo (Lumini et al., 2010; Oehl et al., 2010).

En este sentido hay evidencia de que la funcionalidad de los HMA en términos de *infectividad* (capacidad de los propágulos fúngicos para colonizar las raíces de una planta hospedera) y *efectividad* (capacidad que tienen los hongos micorrícicos para influir favorablemente en el crecimiento y adecuación de una especie vegetal con la que están asociados, en comparación con otra que crece en ausencia de ellos) disminuyen a medida que el uso de la tierra se intensifica (Trejo et al., 2016, Figura 2). Esta disminución en la riqueza y función de los hongos es atribuida a la presión selectiva que ejerce la conversión a sistemas agrícolas (Hendrix et al., 1995), la

labranza (Roldán et al., 2007), fertilización (Bhadalung et al., 2005) y aplicaciones de fungicidas (Oehl et al., 2004), además de los periodos de sequía que se han incrementado.

Se ha demostrado que la sequía y la introducción de una especie exótica de micorriza arbuscular (*Rhizophagus irregularis*) que es ampliamente vendida como inóculo comercial, altera el ensamble de las especies de hongos nativos con consecuencias para la relación simbiótica (Symanczik et al., 2015). Aunque existe una amplia diversidad genética y funcional de especies de HMA (Bever et al., 2001; Helgason y Fitter, 2009), y cada especie responde de manera diferente a la alteración del suelo, el conocimiento sobre su funcionalidad es incompleto.

Las prácticas agrícolas intensivas modernas son evidentemente una amenaza para los HMA, como lo indican los estudios en agroecosistemas, a mayor gradiente de perturbación, menor colonización (Helgason et al., 1998; Douds y Millner, 1999; Vega-Frutis et al., 2018). En general, estos estudios han indicado que no solo la riqueza y abundancia de especies de HMA disminuye con la intensificación agrícola, también la colonización de las raíces, la eficiencia de los hongos para transportar nutrientes minerales hacia sus plantas hospederas y, por lo tanto, la promoción del crecimiento de las plantas (Helgason et al., 1998; Douds y Millner, 1999; Vega-Frutis et al., 2018).

A pesar del importante papel de los HMA en los ecosistemas terrestres, la relación entre su diversidad taxonómica y funcional es poco conocida (Krüger et al., 2009). Un aspecto importante en los estudios ecológicos, es la determinación de su infectividad y efectividad (Smith y Smith, 2011a, b). Estas variables proporcionan información sobre la actividad de los HMA en relación con su capacidad para desarrollar una simbiosis funcional.

Estudios de los HMA en cultivos

En México, los estudios sobre HMA han evaluado las respuestas ecofisiológicas y ecológicas de las plantas. Sin embargo, los aspectos taxonómicos y los estudios *in*

situ, así como su papel en los ciclos biogeoquímicos o en relación con la fertilidad del suelo, la productividad, el secuestro/depósito de carbono, la diversidad de plantas y su uso como bioinoculantes han recibido menos atención (Montaño et al., 2012).

El alto número de ecosistemas y diversidad de plantas reportados para México sugiere que este país está poco explorado como un *hotspot* (sitios con una alta diversidad) de HMA. Por lo tanto, existe una necesidad urgente de ampliar las investigaciones sobre este grupo de hongos a una mayor cantidad de (agro)ecosistemas, incluyendo una mayor cantidad de estados, dado que existe un sesgo en los estados del país y en los ecosistemas que han sido estudiados (Montaño et al., 2012).

Para México existe poca información sobre el cambio de uso de suelo y su efecto sobre la viabilidad de los HMA. Por ejemplo, Guadarrama et al. (2008) realizaron un estudio en Nizanda, Oaxaca, donde reportaron que en un bosque secundario (por eliminación de la vegetación original para la agricultura) después de 27 años de abandono, más del 40% del micelio extrarradical no fue viable y alrededor del 64% de las esporas tenían algún tipo de daño, comparado con un bosque recientemente deforestado. Con ello también demostraron que la disminución en los propágulos fúngicos depende del tiempo de deforestación.

Recientemente, Trejo et al. (2016) en Los Tuxtlas, Veracruz, mostraron que el número de esporas de HMA fue mayor en un sitio de pastoreo comparado con un cultivo de milpa, y aunque la viabilidad de las esporas fue mayor en el cultivo de milpa, esto no se reflejó en los porcentajes de colonización, los cuales fueron mayores en el sitio de pastoreo. El mayor número de esporas y porcentaje de colonización en el sitio de pastoreo probablemente se debió a la introducción de especies de pastos altamente micotróficos (Trejo et al., 2016). En estudios con papaya, Sangabriel-Conde et al. (2010) encontraron que el tipo de manejo (alta, mediana, y baja tecnología) afectó negativamente los propágulos fúngicos, al encontrar menor porcentaje de colonización micorrícica y menor número de propágulos fúngicos en la parcela con manejo de alta tecnología.

En general, diversos estudios reportan la infectividad y efectividad de los HMA en plántulas de aguacate bajo condiciones de invernadero, reportando especies potenciales para producir inoculantes que mejoren el desempeño y la resistencia de las plantas a los patógenos (Banuelos et al., 2013, Carreón-Abud et al., 2013, Castro et al., 2013, Carreón et al., 2014, Bañuelos et al., 2017, Tabla 1).

En el estado de Michoacán, las plantaciones de aguacate han sido establecidas exitosamente en regiones de bosque templado desde 1950, además de las antiguas conversiones para establecer maíz. Sin embargo, la región de aguacate se expandió rápidamente y en los últimos años se ha alentado a los agricultores a convertir más tierras templadas de pino y roble en plantaciones de aguacate potencialmente rentables (INIFAP, 2009). El cambio de uso de suelo también puede acelerar la erosión de la tierra y la degradación, ya que muchas de las nuevas plantaciones se establecen en laderas empinadas y la pérdida de diversidad de plantas puede dificultar las combinaciones compatibles de plantas y hongos (Sanders et al.,1998). Sin embargo, el efecto que tiene el cambio de uso de suelo (a cultivos de aguacate) sobre la diversidad de microorganismos presentes, a mediano y largo plazo, ha sido pobremente documentado.

Si bien, en otros estados y en el resto del mundo se ha estudiado cómo el cambio de uso de suelo en diferentes plantas y cultivos ha afectado la producción de propágulos fúngicos. Nayarit siendo uno de los principales productores de aguacate a nivel nacional, carece de investigación básica sobre su interacción con los HMA, esto a pesar de que la condición asimbiótica para la mayoría de las plantas en ecosistemas naturales y agroecosistemas es rara. El entendimiento de las consecuencias que tiene el cambio de uso de suelo sobre los patrones de relación hongo-suelo-planta son un desafío para entender el rol de estos hongos en el funcionamiento de los (agro)ecosistemas y su posible aplicación en programas de restauración y reforestación. Por lo tanto, los objetivos y las hipótesis de este estudio son:

OBJETIVOS

General

Determinar la infectividad y efectividad de los hongos micorrícicos arbusculares en parcelas de aguacate con y sin manejo nutricional.

Específicos

1. Estimar en campo la infectividad de los HMA en árboles de aguacate en ambas parcelas.
2. Estimar la efectividad e infectividad de los propágulos fúngicos en plantas de aguacate sembradas en sustrato con diferente manejo nutricional.

HIPÓTESIS

1. La infectividad (porcentaje de colonización intrarradical) presente en la parcela sin manejo agrícola será mayor comparada con la parcela que recibe tratamiento químico y orgánico.

2. Las plantas de aguacate inoculadas con los propágulos fúngicos (esporas, micelio extrarradical y fragmentos de raíz colonizados) provenientes de la parcela con tratamiento químico y orgánico tendrán una menor colonización y desempeño comparado con las plantas inoculadas con los propágulos fúngicos de la parcela sin manejar.

Tabla 1 Resumen de estudios realizados en *Persea americana* y los HMA en México.

	Estudio	Tipo de inóculo	Condiciones	Estado	Cita
INFECTIVIDAD Y EFECTIVIDAD	Los inóculos fueron probados en suelo esterilizado y sin esterilizar, donde pudieron observar que hay una posible compatibilidad hongo-planta, probando que esta interacción brinda un incremento en el peso de la biomasa aérea. Además, probaron la importancia de la esterilización antes de inocular con HMA ya que la microbiota nativa puede interferir con el establecimiento de HMA introducidos.	Especies monoxénicas. Consorcio comercial. Control no micorrizado.	Invernadero	Veracruz	Banuelos et al., 2013
	La técnica de minirizotrón, mostró ser efectiva en la propagación de cultivos multiespecíficos o monospóricos *in vivo.*	Esporas provenientes de dos parcelas de aguacate con diferente manejo agrícola.	Campo / Invernadero	Michoacán	Carreón-Abud et al., 2013
	Las plantas criollas raza mexicana inoculadas con HMA presentaron un mayor desarrollo que las plantas testigo, sin diferencias significativas entre los dos consorcios de HMA utilizados. Además, las plantas micorrizadas mostraron mayor capacidad fotosintética.	Consorcios comerciales. Control no micorrizado.	Invernadero	Michoacán	Castro et al., 2013
	Evaluaron el desempeño de 12 inoculantes de HMA, utilizando plantas de 60 días de edad, recientemente injertadas. Observaron que hay especies que potencializan el crecimiento de la parte aérea, otras la subterránea o ambas, por lo que es importante inocular con un conjunto de especies. Después de 6 meses de haber sido inoculadas, mostraron diferencias evidentes respecto a las especies foráneas y el control.	Especies nativas. Especies foráneas. Consorcio comercial. Control no micorrizado.	Invernadero	Michoacán	Carreón et al., 2014
	Observaron que los HMA son una alternativa para aumentar la absorción de P y reducir el excesivo uso de fertilizante fosfatado. Bajas concentraciones de fertilizante fosfatado e inoculación con HMA, promueve mayor incremento en las variables de crecimiento.	Consorcio comercial. Especie *Pacispora scintillans.* Control no micorrizado.	Invernadero	Veracruz	Bañuelos et al., 2017

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CAPÍTULO II

Short communication

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

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SUMMARY

Mexico is the principal avocado producer worldwide. However, this has increased the use of agrochemicals and thus has reduce the biotic interactions belowground such as arbuscular mycorrhizal fungi. Arbuscular mycorrhizal 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, México. In agreement with other studies, our results showed that chemical fertilizer had negative effects on the intraradical fungal structures, and the orchard that receives agrochemicals had lower mycorrhizal colonization. We discussed the implications that the agrochemicals have on arbuscular mycorrhizal symbiosis.

KEYWORDS / Agroecosystems / fungal propagules / land-use change / symbiosis /

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

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

RESUMEN

México es el principal productor de aguacate en el mundo. Sin embargo, esto ha incrementado el uso de agroquímicos, y por lo tanto, han disminuido las interacciones bióticas del suelo como la micorriza arbuscular. Los hongos micorrizógenos arbusculares pueden contribuir a la restauración de los ecosistemas degradados. Por lo tanto, nosotros 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, nuestros resultados mostraron que el fertilizante químico tuvo efectos negativos en las estructuras fúngicas intrarradicales, y la parcela que recibe agroquímicos tuvo la menor colonización micorrícica. Nosotros

discutimos las implicaciones que los agroquímicos tiene sobre la simbiosis micorrícica arbuscular.

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

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

RESUMO

O México é o principal 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, nós 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, nossos resultados mostraram que o fertilizante químico teve efeitos negativos nas estruturas fúngicas intra-radicais, e a parcela que recebe agroquímicos apresentou a menor colonização micorrízica. Discutimos as implicações que os agroquímicos têm na simbiose micorrízica arbuscular.

Introduction

Anthropogenic activities such as agricultural intensification, deforestation, urban expansion and industrial activities are some of the main global causes of above- and belowground biodiversity decline in the 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 negatively affecting particle aggregation, nutrient leaching, nutrient cycling, and belowground 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 (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, 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 Nayarit is the fourth national producer with a total of 49,245.79 tons produced in the year 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*., 2012; Castro *et al*., 2013; Carreón-Abud *et al*., 2015). However, little attention has been paid in 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 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 coordinates of each orchard are presented in Table I. The orchard 1 does not receive chemical nutrients, while the orchard 2 receives a combination of chemical (N, P, K, Ca, Zn, B, and Mg, ~4kg/tree per year) and organic (chicken manure, 30kg/tree per year) nutrients.

Arbuscular mycorrhizal colonization measurements

In each orchard, we collected fine roots from 30 random reproductive avocado trees in two plots of 50 x 50 m (15 trees per plot), and we 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 – 1.5 m around the tree trunk. The roots were placed in ethanol (50% v/v), afterwards they were 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 ~1.5 cm long) from each tree. Each root fragment was examined at three equally spaced points under a light microscope at

100x and 400x total magnification, using the cross-hair intersection method (McGonigle *et al*., 1990).

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_4 pH 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, total carbon (TC), total hydrogen (TH) and total nitrogen (TN): %. Carbon/Nitrogen (C/N). Clay, silt and sand: %.

Soil chemistry

Five samples of soil per plot (10 samples per orchard) were collected. The soil was homogenized, and we took a subsample of ~1 kg per orchard for the chemical analyses

(Table I). Chemical soil parameters were determined according to the Mexican Official Standard NOM-021-PROJ-RECNAT-2000 (SEMARNAT, 2002), and were carried out in the Laboratory of Soil Analyses of the Ecology Institute A. C. (INECOL).

Data analyses

We used a nested ANOVA model 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 by Neperian logarithm. To 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, we used the BSC as 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 ± 4.15 cm), compared with orchard 1 (73.88 ± 4.93 cm). Opposite, the percentage of colonization by hyphae (82.25 ± 4.30 cm, 63.01 ± 4.75 cm) vesicles (76.76 ± 4.60 cm, 60.88 ± 4.89 cm) and arbuscules (46.04 ± 4.10 cm, 23.19 ± 3.51 cm) were higher in orchard 1 compared to orchard 2 respectively, and these differences were statistically significant (Table II). Although the trees showed differences in their BSC between orchards, the BSC did not affect the fungal intraradical structures (Table II).

Table II Summary of statistical results of Nested ANCOVA models for fungal structures analyzed in avocado trees.

Source of variation		Hyphae (%)		Vesicles (%)		Arbuscules (%)	
	df	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.

Our study showed that the trees growing in orchard 2 had the lowest AM colonization for all quantified fungal structures. Also, this orchard had, overall, the biggest trees, and these results agree with our initial prediction. Our 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; Trejo *et al*., 2016; Vega-Frutis *et al*., 2018). For instance, Vega-Frutis *et al*. (2018) found that the frequency of AM colonization in the wild papaya decreased when the disturbance increased. The same pattern was observed by Oehl *et al*. (2003), they observed that the frequency of AM colonization was lower in monocropping. In the same way, 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), the 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 the 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 belowground microorganism (Gagic *et al*., 2017).

Several studies have shown that increasing soil P availability significantly reduce the P uptake pathway mycorrhizal fungi (Konvalinkova *et al*., 2017). In our study, the orchard 1 had the highest AM colonization, but also total soil P. We did not quantify the soil P available, therefore, it is probable that the amount of P available for avocado trees might be lower than orchard 2. This could explain the high AM colonization in the orchard 1. In addition, we 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, Carreón-Abud *et al*. (2012) observed percentages of mycorrhizal colonization of approximately 80 to 100%. However, plant species show intraspecific variation in root morphology and 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, 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. Thus, our study is the first showing that management practices decrease AM colonization. The AM fungi can reduce the use of agrochemicals, promoting soil health, plant performance and quality in the

agroecosystems. Therefore, more investigations of the interactive effects of management practices and mycorrhizal symbiosis in avocado crops are 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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CAPÍTULO III

Original paper

Chlorophyll fluorescence, root traits and biomass in mycorrhizal plants of *Persea americana* under different soil agricultural management

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**** Formato de la revista Biology and fertility of soils**

Abstract

Arbuscular mycorrhizal (AM) fungi constitute a group of root obligate biotrophs that exchange mutual benefits with about 80% of land plants including most crops. Several papers have been written about the role of mycorrhizas in agroecosystems with a view to sustainable agriculture. In this study, we investigated the effectivity (chlorophyll fluorescence, root traits and biomass) and infectivity (percentage of AM colonization) in avocado plants under a factorial experiment: 1) the origin of seeds from two landrace trees, 2) origin of soil from two avocado orchards with and without nutritional management, and 3) the AM inoculum isolated from the same avocado orchards. Overall, the inoculated plants showed a mycorrhizal plant benefit in the parameters evaluated compared with non-mycorrhizal plants. Although the mineral soil nutrients and origin of seeds influence these results. Regarding to infectivity, the plants growing in soil from orchard without nutritional management and inoculated with fungal propagules from the same orchard had greater percentages of AM colonization, suggesting that there is a local adaptation between these fungi and its host plants. We discuss our findings in the context of soil mineral nutrients. This study contributes to the understanding of the relationship between the avocado plants, an important cultivated tree for the humans in the worldwide, and its mycorrhizal symbionts.

Keywords

Arbuscular mycorrhizal fungi, chlorophyll fluorescence, root colonization, root traits, Nayarit.

Introduction

Soil microorganisms such as arbuscular mycorrhizal (AM) fungi play a key role linking the plant roots and soil mineral nutrients. AM fungi belonging to subphylum Glomeromycotina (Spatafora et al. 2016) are an ancient lineage of obligate biotrophs (they need a living host plant during their life cycle) that colonize the roots of most terrestrial plant species (around 80% of plant species and 92% of plant families, Wang and Qui 2006). During this symbiosis, the plant supplies their associated fungi with a microhabitat and carbohydrates for fungal survival and growth. In exchange, AM fungi form extensive networks of hyphae in the soil, foraging for inorganic nutrients and water more efficiently than roots and transfer them in symbiotic specific fungal structures (arbuscules) inside the roots (Smith and Read 2008). In addition, these fungi can act as a bioprotector of biotic (Gehring and Bennett 2009) and abiotic (Arafat et al. 2016) stresses, and consequently the plants improve their nutritional status. Arbuscular mycorrhiza, also, provide several ecosystem services, for instance, increase soil adherence, soil stability, and water retention (Gianinazzi et al. 2010). Therefore, they are considered natural biofertilizers (Berruti et al. 2016).

During the last years, the number of articles focused about the role of mycorrhiza in (agro)ecosystems have been increased (Naher et al. 2013; Berruti et al. 2016; Rillig et al. 2016; Ryan and Graham 2018, Rillig et al. 2019). The reason is reducing the dependence of agrochemicals products, and decrease the negative effects on the soil biodiversity and soil biochemistry processes, in order to safeguard human and environmental health. Although farmers have not adopted the utilization of AM fungi so far, several studies have shown the positive effects of AM fungi from a range of cereals, crops, fruits and vegetables, and some generalizations can be made. For instance, plant parameters such as dry weight, nitrogen and phosphorous uptake, plant height, water content, chlorophyll, and higher resistance to pathogens increase in mycorrhizal plants compared with non-mycorrhizal plants (Zhu et al. 2011; Naher et al. 2013; Shamshiri and Fattahi, 2016; Alam et al. 2019). Additionally, the use of native AM fungi has shown more benefit to the plants than exotic AM inoculum, although most studies have been done under greenhouse conditions (Berruti et al. 2016).

The plant-fungi is a complex biotic interaction, and although many crops have positive mycorrhizal benefit, factors such as soil nutrients availability, climatic variables, soil biogeochemistry, and the symbiont's identities are known to affect the outcome of the mycorrhizal symbiosis. For instance, high concentrations of chemical fertilizers may favor plant genotypes that are less tolerant or capable of associating with microbial symbionts. Kiers et al. (2007) found that older cultivars of *Glycine max* (soybean) obtained a higher benefit from mixed N_2-fixing bacteria than newer cultivars, perhaps due to artificial selection. Newly, Ryan and Graham (2018) concluded that there is little evidence that farmers should consider the abundance and diversity of AM fungi when they managing their crops, because only few studies have reported positive impact of AM colonization on crop yield. But against, Rillig et al. (2019) pointed up that there is evidence of positive AM fungal effects from a range of crops but is important include variables that express the mycorrhizal plant benefit.

Recently has been suggested that root traits such as specific root length, root branching ratio, and root diameter are important determinants of mycorrhizal colonization (Kong et al 2014; Wen et al. 2019). In general, species with thicker roots, lower root branching ratio, and lower specific root length support greater AM colonization (Kong et al. 2014; Vega-Frutis et al. 2015; Wen et al. 2019) suggesting higher mycorrhizal dependency. Given that the AM fungi received carbohydrates for their host plants, the chlorophyll fluorescence has been used as a non-invasive measurement to evaluate the physiological condition of plants especially under stress conditions (Baker 2008; Zhu et al. 2010; Zhu et al. 2011; Vega-Frutis et al. 2014). For instance, it has been observed that *Pistacia vera* plants under normal conditions and saline stress, as well as *Macadamia tetraphylla* plants under water stress, presented an increase in the quantum yield of photosystem II and consequently an integral increase in photosynthesis when these were inoculated with AM fungi compared to non-mycorrhizal plants (Yooyongwech et al. 2013; Shamshiri and Fattahi 2016). However, intraspecific variation in plant parameters (e.g., specific root length, root branching ratio, photosynthesis, water status), and fungal colonization have been observed (Wen et al. 2019). Nevertheless, most studies on root traits are done in

herbaceous species but research on cultivated species is lacking (Xue-Guang and Ming 2013; Maherali 2014; Vega-Frutis et al. 2015; Martín-Robles et al. 2017; Valverde-Barrantes et al. 2017).

One of the most important cultivate plants for the humans in the worldwide is the avocado, *Persea americana* (Rendón-Anaya et al. 2019). The avocado is a fruit tree from Mesoamerica and Central America (Barbosa-Martín et al. 2016). Mexico is the largest producer of avocado worldwide with approximately 30% of global production, and the state of Nayarit is positioned in the fourth place of harvest of this fruit (SIAP, 2018). Consequently, the land cultivated area has increased, in the same way that the use of fertilizers, insecticides and herbicides, leading to soil erosion and deforestation of natural forest areas (Barsimantov and Navia 2012). This change in the land-use decrease soil fungal propagules (spores, extraradical hyphae and root pieces colonized by AM fungi). Thus, affecting negatively the capacity of AM propagules to colonize the roots of their host plants (infectivity). Therefore, affecting directly and indirectly the yield of crops and the soil health (Moorman and Reeves 1979; Smith and Smith 2011; Rillig et al. 2019).

Previous studies have reported that the avocado is highly dependent on the AM fungi (Gómez et al. 2012; Rivera et al. 2016). The avocado trees with AM fungi obtain benefits in terms of growth and development, better nutrient absorption, and greater photosynthetic capacity (Castro-Alvarado et al. 2013; Carreón et al. 2014; Bañuelos et al. 2017). Therefore, it is a priority to study the interaction between the plants and their symbionts in natural and protected areas (Montaño et al. 2012). The management practices are key for sustainable agriculture and understanding the processes that occur belowground is a challenge to agroecosystems in agreement with the biodiversity conservation, and the rehabilitation of them (Rillig et al. 2019). In the present study, we evaluated the effectivity (chlorophyll fluorescence, root traits and biomass) and infectivity (percentage of AM colonization) in avocado plants under a factorial experiment: 1) the origin of seeds from two trees of landrace avocado, 2) the soil origin from two avocado orchards with and without nutritional management, and 3) the AM inoculum isolated from the same avocado orchards. Therefore, the aim of this study

was evaluated whether the combination of factors affect the chlorophyll fluorescence, root traits and biomass in avocado plants. Since several studies have reported that the fertilization tend to negatively affect AM fungal propagules and belowground biotic interactions (e.g. Oehl et al. 2010; Trejo et al. 2016; Vega-Frutis et al. 2018), we predict greater infectivity and effectivity in avocado plants growing in soil and AM inoculum without nutritional management than another one.

Materials and methods

Study plant

Avocado (*Persea americana*, Lauraceae) is a fruit tree native to Mesoamerica and Central America, but is currently grown worldwide (Barbosa-Martín et al. 2016). In this study, mexican landrace avocado seeds were used. Around the world, this and other varieties of landrace avocado are used as rootstock of the commercial varieties such as Hass, because they are more resistant to pests and diseases (Sánchez-Pérez, 1999), and are directly associated with soil microorganisms. Mainly, the mexican landrace avocado is resistant to root diseases (Rincón-Hernández et al. 2011).

Experimental setup

Seed and soil material collection

In August 2017, we collected 400 ripe fruits from two landrace avocado trees. The avocado trees were localized inside of Agriculture Faculty, Autonomous University of Nayarit (104.89085° W, 21.4288333° N). 170 fruits were chosen at random from each tree and the fruits were enumerated and weighed, once they reached their edible maturity, the seeds were extracted, weighed and stored in brown paper bags at room temperature for one week.

In September 2017, soil was collected from two avocado orchards located in the State Reserve Sierra de San Juan, in Xalisco, Nayarit, Mexico. Orchard 1 (104.94894º

W, 21.40142º N) is characterized for being without pest, disease and soil nutrition management (Luna-Esquivel, personal communication, September 2017). While in the orchard 2 (104.93842º W, 21.39864º N) the avocado trees are fertilized with poultry manure (30 kg/plant per year, for three years ago) during the dry season, and with chemical fertilizer mixture. The amount of chemical fertilizers varies according to the foliar analyses and soil fertility, but the mixture of N, P, K, Ca, Zn and Mg (average application of this mixture 4 kg/plant per year) has two annual applications: first application at the end of July, and second application at the end of August or beginning of September. In this orchard, a micro-sprinkler irrigation is applied once per week (60 L per plant, Luna-Esquivel, personal communication, September 2017).

In each orchard, two plots of 50 m x 50 m were placed. Inside each plot, five random points were selected. The soil was collected with a shovel at a depth of 20 cm, and approximately 150 kg of soil were collected per orchard. The samples per orchard were homogenized and divided into three parts: 1) 1 kg per orchard for physical-chemical analyses of the soil, 2) 1.5 kg per orchard to be used as inoculum, and 3) the rest of the soil was sterilized for the experiment of infectivity and effectiveness.

Germination

Avocado seeds were individually submerged in plastic cups (~200 ml volume) with 1.5% NaClO for 5 minutes, after which time they were transferred to plastic cups (~200 ml volume) with purified water for 5 minutes (Hernández-Cuevas and García-Sanchez 2008). Later, the tip of each seed was cut and these were transferred into the germination trays (Hydro-Environment of 32 cavities), previously washed and disinfected with 10% sodium hypochlorite, dried and the orifices were covered with a piece of paper towels (Sanitas® Kimberly - Clark). The cavities were filled with a mixture containing autoclaved commercial soil and sand (1:1). Commercial soil and sand were heat sterilized in an autoclave (BIOBASE®) for 90 minutes at 125 °C (1.2 Mpa), after 24 hours we done a second sterilization under the same conditions mentioned above with the aim to kill all AM propagules and have plants free of arbuscular mycorrhizal

fungi. During the first 10 days, the seeds were irrigated daily with well water and after that every two days.

Transplant

After 45 days of the germination, 60 randomly plants of each tree were weighed (initial fresh total biomass). Thus, in the beginning of the experiment the plants showed significant differences (60.710 ± 1.518 g from tree 1 and 50.260 ± 1.229 g from tree 2, $F_{1,118} = 27.695$, $P < 0.001$; see statistical analyses). Later, the plants were transplanted into black bags filled with ~2 kg of sterilized soil coming from the orchards (Banuelos et al. 2013). The soil was sterilized twice for two consecutive days as mentioned above. Before being used, the soil was allowed to aerate for 72 hours to eliminate biotic communities, but to retain the abiotic features of the soil (Johnson et al. 2010). All soil physical-chemical analyses were carried out in the Soil Analysis laboratory of the Institute of Ecology A.C (INECOL) in agreement with the Official Mexican Norm PROY-NOM-021-RECNAT-2000 (SEMARNAT 2002). The physical-chemical soil parameters are presented in the Table S1 and Fig. 1.

The experiment was initiated in October 2017, and we performed a factorial experiment with three factors: origin of seed either from tree 1 (T1) or tree 2 (T2), origin of soil from orchard 1 or orchard 2 (O1 and O2), and origin of AM inoculum from orchard 1 (AMO1), orchard 2 (AMO2) or without inoculation (NM). We had 10 replicates of each treatment combination, with 120 plants.

The mycorrhizal inoculum consisted of 20 g of unsterilized soil per plant from each orchard. The 20 g of fungal propagules were constituted by approximately 332 ± 42.394 (mean ± standard error) and 402 ± 25.438 spores from O1 and O2 respectively. The spores were extracted used the wet sieving and decanting method (Gerdemann and Nicolson 1963) in three replicates per orchard and counted at 35x magnification with a stereomicroscope (LEICA EZ4). For extraradical mycelium, we used 5 g of dry soil from three samples per orchard, and the method of Sylvia (1992) was used to extract the mycelium. The length of extraradical mycelium was estimated using a grid

in the eyepiece of light microscope (ZEISS Primo Star) at 400x of magnification total in agreement with the formula of Brundrett et al. (1994). On average, 2.895 ± 0.143 cm g^{-1} soil and 2.672 ± 0.236 cm g^{-1} soil were estimated from O1 and O2 respectively. In addition, we separated and weighted all potentially colonized root fragments from 5 g of soil samples per orchard, and 25.900 ± 4.696 mg and 28.766 ± 2.907 mg from O1 and O2 respectively were obtained. For non-mycorrhizal plants (NM), 20 g of sterilized soil was used (Johnson et al. 2010). During the transplant, 20 g of inoculum was placed directly on the root. Additionally, all plants received 15 ml of a soil microbial suspension filtered through a 0.8 µm nitrocellulose membrane Millipore filter. The microbial suspension was prepared from the unsterilized soil from each orchard in order to partially return the microflora to the sterilized soil.

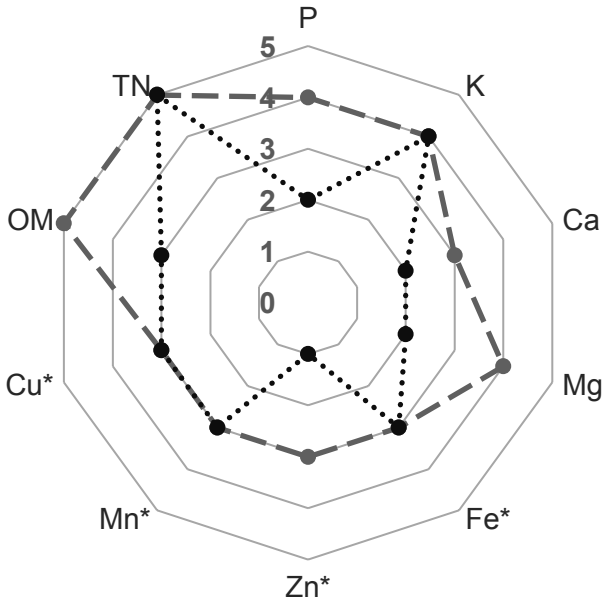

Fig. 1 Summary of sterilized soil parameters quantified from orchard 1 (O1) and orchard 2 (O2) located in the State Reserve Sierra de San Juan, Nayarit, Mexico. Interpretation: 1) very low or deficient, 2) low or marginal, 3) medium or adequate, 4) high and 5) very high (in accordance with Castellanos et al. 2000). The dashed line (---) represent the properties of orchard 1 and the dotted line (⋯) represent the properties of orchard 2. Determinations with asterisk (*), means that its minimum scale is 1) and its maximum scale is 3).

After the plants were assigned to the treatments, the pots were randomly placed in a nursery garden in the Agriculture Faculty, Autonomous University of Nayarit. To avoid potential effects due to the arrangement of the pots in the nursery garden, these were rotated every two weeks during the 129 days of the experiment (end of the experiment March 2018), and watered every third day with well water. In addition, in November 2017 and February 2018, AgroIQC® avermectin (3 ml/L H_2O) was applied once per month because the presence of the red spider (*Tetranychus urticae*) was detected.

Plant measurements

Chlorophyll fluorescence

After 126 days, chlorophyll fluorescence was measured using Li-Cor 6800 portable photosynthesis measuring system (Li-Cor, Inc., Lincoln, Nebraska, USA). Before to measure the chlorophyll fluorescence, the leaves were darkening for 30 min (Goltsev et al. 2016). Then, we recorded, the minimal fluorescence (F_0) and maximal fluorescence (F_m) applying three pulses of saturating light from 1000 µmol m^{-2} s^{-1} every 5 s. These parameters were recorded in one fully expanded apical leaf per plant, and the pulses of saturating light were averaged. The variable fluorescence yield (F_v) was calculated as $F_v = F_m - F_0$, and the ratio of variable to maximum fluorescence (F_v/F_m) was calculated as maximum quantum efficiency of photosystem II (Yooyongwech et al. 2013; Goltsev et al. 2016). Chlorophyll fluorescence measurements provide a sensitive method to evaluate plant physiology, with optimal values of around 0.8 (± 0.05) measured in most plant species including cultivate plants. Values lower than 0.75 are considered an indicator of damage caused by abiotic stresses to the photosystem II (photoinhibition, Maxwell and Johnson 2000; Baker 2008; Márquez 2017).

Root traits

During the harvested (March 2018), a subsample of roots per plant was collected. Root segments including first and second-order roots were washed with gently running water, and a high-resolution picture was taken. Afterward, the root segments were oven-dried at 60 °C for three days. Later, the root diameter (RD) of all first-order roots, and the root length were measured using the complementary software SmartRoot from ImageJ software (Lobet et al. 2011). Additionally, the specific root length (SRL) was estimated by dividing the root length by the root dry biomass, and the root branching ratio (RBR) was estimated as the number of first-order roots relative to the number of second-order roots. These root traits are expected to influence the mycorrhizal colonization (Chen et al. 2013; Kong et al. 2014; Liu et al. 2015).

Plant biomass

The experiment lasted 129 days, simulating the time in which the avocado plants are transplanted to the field. The plants were harvested and weighed to obtain the final total fresh biomass, and the relative growth rate (RGR) was calculated as [ln (final fresh biomass) - ln (initial fresh biomass)/129 days]. Subsequently, the aboveground (AB, leaves and shoots) and belowground (BB, roots) biomasses were separated, placed in brown paper bags, and oven-dried at 60°C for three days. The root/shoot ratio (R/S) was calculated as belowground dry biomass/aboveground-dry biomass.

Fungal measurements

During the harvest a subsample of fine roots of all plants were collected to estimate the percentage of root colonized by AM fungi. The roots were processed according to the method of Koske and Gemma (1989) and stained with trypan blue (0.05%). Subsequently, the intraradical colonization by hyphae, vesicles and arbuscules was quantified on 15 root segments of approximately 15 mm long per plant and were fixed in polyvinyl-lacto-glycerol (PVLG) on permanent slides. Each root fragment was

examined at three equally spaced points under an optical microscope (ZEISS Primo Star) at 100x total magnification. The presence/absence of each intraradical fungal structure across the equator from each field of view under the microscope was registered. Positive counts were summed and weighted by the total number of fields of view observed and multiplied by 100 (McGonigle et al. 1990).

Statistical analyses

All statistical analyses were performed using the R statistical language (R Development Core Team, 2017). Before to running the tests, we performed a graphical data exploration to evaluate the best potential models with which to analyze each type of response variable. For all the models, we verified whether the residuals were normally distributed, the variances homogeneous, and when it was necessary the variables were transformed (see below).

Plant measurements

To test for differences in the initial fresh biomass between the origin of the seeds (tree 1 and tree 2), we used one-way ANOVA. To meet model assumptions, the response variable was square root transformed.

The effect of the origin of seeds (T1 and T2), origin of soil (O1 and O2), origin of AM inoculum (AMO1, AMO2, and NM) and the interaction between these three factors on minimal fluorescence (F_0), maximal fluorescence (F_m), the ratio of variable to maximum fluorescence (F_v/F_m), specific root length (SRL), root branching ratio (RBR), root diameter (RD), relative growth rate (RGR), aboveground biomass (AB), belowground biomass (BB), and root/shoot ratio (R/S) were tested with analysis of covariance (ANCOVA), the initial fresh biomass (IFB) was used as a covariate. To meet the model assumptions, the response variables of F_0, F_m and SRL were transformed with square root, F_v/F_m, RBR, RD, RGR, AB, BB, and R/S were transformed with rank ("average" method). Significand differences between the levels of origin of AM

inoculum (AMO1, AMO2, and NM) were tested with a *posteriori* contrast based on *t*-test using the *lsmeans* library (Lenth 2016), which calculates the means of least squares (expected marginal means) for specific factors or combinations of factors in a linear model. When the triple interaction was significant, we tested the effect of origin of seeds (T1 and T2), origin of soil (O1 and O2), and the interaction between these two factors separately from the origin of AM inoculum (AMO1, AMO2, and NM).

Fungal measurements

We also used an ANCOVA to explore differences in the percentages of root colonized by hyphae, vesicles and arbuscules among the origin of seeds (T1 and T2), origin of soil (O1 and O2), AM inoculum (AMO1 and AMO2) and the interaction between these factors. The initial fresh biomass was used as covariable. To meet the assumptions of the model, the percentage of vesicles was transformed with range ("average" method).

Results

Plant measurements

Chlorophyll fluorescence

None of the factors evaluated (origin of soil, seeds and AM inoculum) affected the variables F_0 and F_v/F_m (Table 1). But, F_m was affected by the origin of the soil (Table 1). The plants growing in the soil from O1 had a higher F_m than the plants that grew in the soil from O2 (Table 2).

The interaction between origin of seeds and the covariate had a significant effect on the F_0 and F_v/F_m. The F_0 decreased as they had higher IFB (Fig. 2a), and the plants from T2 had the opposite effect as they had higher IFB (Fig. 2a). The interaction between origin of seeds and the covariate did not significantly affect the F_m (Fig. 2b). The F_v/F_m of avocado plants from T1 improved as they had higher IFB (Fig. 2c). In contrast, the F_v/F_m of avocado plants from T2 decreased as they had higher IFB (Fig.

2c). Additionally, the triple interaction between the origin of the seeds (T1 and T2), origin of the soil (O1 and O2), and origin of AM inoculum (AMO1, AMO2 and NM) significantly affected F_0 and F_V/F_m. However, when we tested the effect of origin of the seeds and origin of soil separately for AM inoculum, the only interaction that persisted significantly was the seed origin with the IFB (F_0: $F_{1,48} = 14.439$, $P < 0.001$ and F_V/F_m: $F_{1,48} = 8.337$, $P = 0.005$).

Table 1 Summary of the statistical results of ANCOVA models for plant parameters analyzed in *Persea americana.*

Source of variation		F_0		F_m		F_v/F_m		SRL		RBR		RD		RGR		AB		BB		R/S	
	df	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P	F	P
T	1	0.056	0.813	0.019	0.889	0.025	0.874	9.160	**0.003**	1.318	0.253	0.114	0.736	25.467	**<0.001**	1.436	0.233	3.697	**0.057**	0.988	0.322
O	1	0.060	0.805	4.964	**0.028**	0.807	0.370	0.864	0.354	0.016	0.897	1.971	0.163	0.005	0.941	14.084	**<0.001**	0.141	0.708	19.937	**<0.001**
In	2	0.220	0.802	0.247	0.781	0.429	0.652	3.841	**0.024**	3.416	**0.036**	0.173	0.840	2.620	0.077	4.136	**0.018**	0.295	0.744	2.866	0.061
IFB	1	1.384	0.242	0.197	0.657	0.731	0.394	1.350	0.247	1.032	0.311	3.530	0.063	5.747	**0.018**	51.281	**<0.001**	117.674	**<0.001**	2.165	0.144
T:O	1	0.055	0.813	0.076	0.782	0.119	0.730	0.542	0.463	1.588	0.210	0.309	0.578	0.206	0.650	4.408	**0.038**	0.362	0.548	7.905	**0.005**
T:In	2	0.533	0.587	1.385	0.254	0.445	0.642	3.625	**0.030**	3.229	**0.043**	1.835	0.164	1.955	0.146	3.783	**0.025**	3.177	**0.045**	7.530	**<0.001**
T:IFB	1	7.508	**0.007**	0.276	0.600	4.413	**0.038**	4.689	**0.032**	2.194	0.141	0.055	0.815	0.709	0.401	2.482	0.088	1.151	0.320	0.878	0.418
O:In	2	0.071	0.931	0.108	0.896	0.007	0.992	0.012	0.987	0.197	0.821	0.294	0.745	1.699	0.187	0.191	0.662	1.134	0.289	3.034	0.084
O:IFB	1	0.178	0.673	0.926	0.338	0.0001	0.990	2.095	0.150	1.883	0.172	2.477	0.118	2.715	0.102	0.164	0.686	1.772	0.186	1.058	0.306
In: IFB	2	2.748	0.068	0.927	0.398	1.661	0.194	3.889	**0.023**	1.054	0.352	0.357	0.700	4.509	**0.013**	3.388	**0.037**	1.419	0.246	4.234	**0.017**
T:O:In	2	3.029	**0.052**	0.036	0.964	3.280	**0.041**	1.583	0.210	1.200	0.305	0.006	0.993	0.521	0.595	0.223	0.800	1.917	0.152	2.085	0.129
Residuals	103																				

T: origin of seeds (T1, T2); O: origin of soil (O1, O2); In: origin of AM inoculum (AMO1, AMO2, NM); IFB: initial fresh biomass; F_0: minimum fluorescence; F_m: maximum fluorescence; F_v/F_m: ratio of variable to maximum fluorescence ; SRL: specific root length, RBR: root branching ratio; RD: root diameter; RGR: relative growth rate; AB: aboveground biomass; BB: belowground biomass; R/S: root/shoot ratio.

Table 2 Means ± standard errors of chlorophyll fluorescence parameters analyzed in *Persea americana*. Different letters indicate statistically significant differences according to t-test contrast ($P < 0.05$) among origin of seeds (tree), origin of soil (orchard) and the origin of AM inoculum (AMO1, AMO2, NM).

Variables	Tree		Orchard		AM inoculum		
	1	2	1	2	AMO1	AMO2	NM
F_0	671.29 ± 54.58	671.21 ± 51.93	657.41 ± 48.92	685.09 ± 57.24	681.82 ± 57.06	676.48 ± 67.73	655.45 ± 70.90
F_m	2917.12 ± 88.35	2934.81 ± 90.58	3078.50 ± 102.83 a	2773.43 ± 68.19 b	2970.39 ± 118.19	2861.24 ± 106.08	2946.28 ± 104.64
F_V/F_m	0.759 ± 0.021	0.759 ± 0.020	0.766 ± 0.022	0.752 ± 0.020	0.750 ± 0.027	0.756 ± 0.026	0.771 ± 0.024

F_0: minimal fluorescence; F_m: maximal fluorescence; F_V/F_m: ratio of variable to maximum fluorescence.

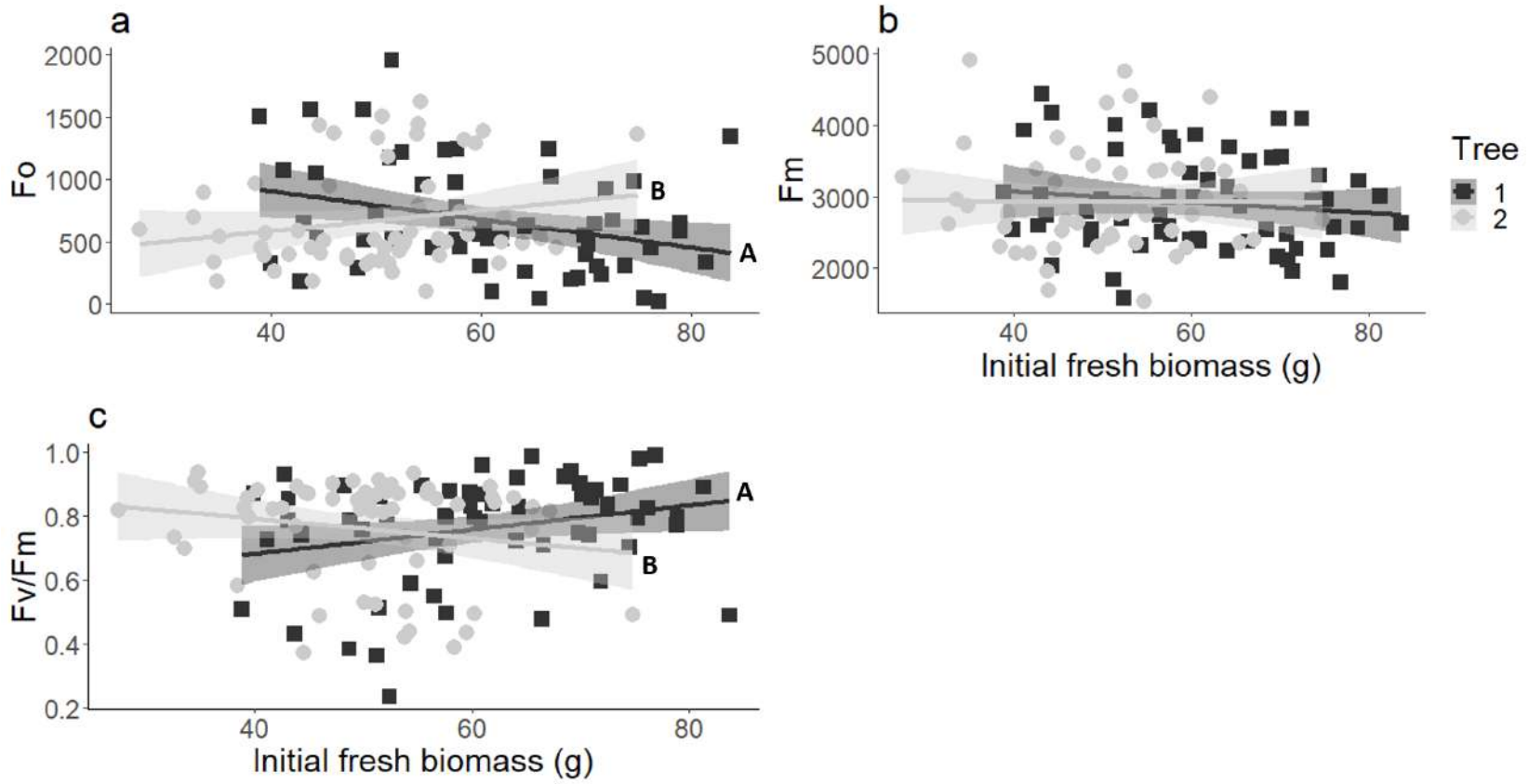

Fig. 2 Interactive effects of origin of seed and the initial fresh biomass (covariate) on (a) minimal fluorescence, (b) maximal fluorescence, and (c) ratio of variable to maximum fluorescence in *Persea americana* plants that grew with seed from tree 1 (black squares) or tree 2 (grey circles). The black (tree 1) and light grey (tree 2) lines represent the regression lines with their respective confidence intervals (shaded). Different letters above the lines indicate statistically significant differences among origin of seeds according to *lsmeans* contrast *P* (<0.05).

Root traits

The origin of seeds and AM inoculum significantly affected the SRL (Table 1). The plants from T2 developed a greater SRL (1921.405 ± 111.355 cm pixel^{-1}) compared with plants from T1 (1517.001 ± 94.905 cm pixel^{-1}). The plants growing without mycorrhizal fungi developed lower SRL (1467.585 ± 114.339 cm pixel^{-1}) than plants growing with inoculum from AMO1 and AMO2 (AMO1: 1789.987 ± 124.446 cm pixel^{-1}, AMO2: 1900.037 ± 143.386 cm pixel^{-1}), with no significant differences between them (p= 0.914, Fig. 3a), and both AM treatment differed of NM (AMO1-NM p= 0.048, AMO2-NM p= 0.013). The RBR only was significantly affected for the AM inoculum. The plants growing without fungi had lower RBR (0.085 ± 0.017 cm pixel^{-1}) compared with the plants from AMO2 (0.094 ± 0.008 cm pixel^{-1}, AMO2-NM p= 0.022), and there were not statistically significant differences between AM treatments explaining the RBR (AMO1-AMO2 p= 0.402, Fig 3b). No statistical differences were detected for the factors origin of seeds, origin of soil and AM inoculum explaining the RD variable.

The interaction between seed origin and AM inoculum significantly affected SRL and RBR, but no RD (Table 1). Avocado plants from T1 and NM treatment, developed lower SRL compared with the plants from T2 (Fig. 3a), and the same pattern was observed for the variable RBR (Fig. 3b) but not for RD (Fig. 3c).

There were statistically significant differences between the variables seed origin and AM inoculum with the covariable (IFB) explaining the SRL (Table 1). Smaller plants developed greater SRL in the AM treatments than plants in the NM treatment (Fig. 4a). The AMO1 treatment was the one who mostly contributed to the development of SRL in larger plants (Fig. 4a). Neither the RBR nor the DR showed significant differences (Fig. 4b and 4c). The plants from T2, which were smallest at beginning of experiment (T2: 50.260 ± 1.229 g, T1: 60.710 ± 1.518 g) developed greater SRL (Fig. 4d). While the RBR and RD were not affected by the interaction between the origin of seed with the covariable (Fig. 4e and 4f).

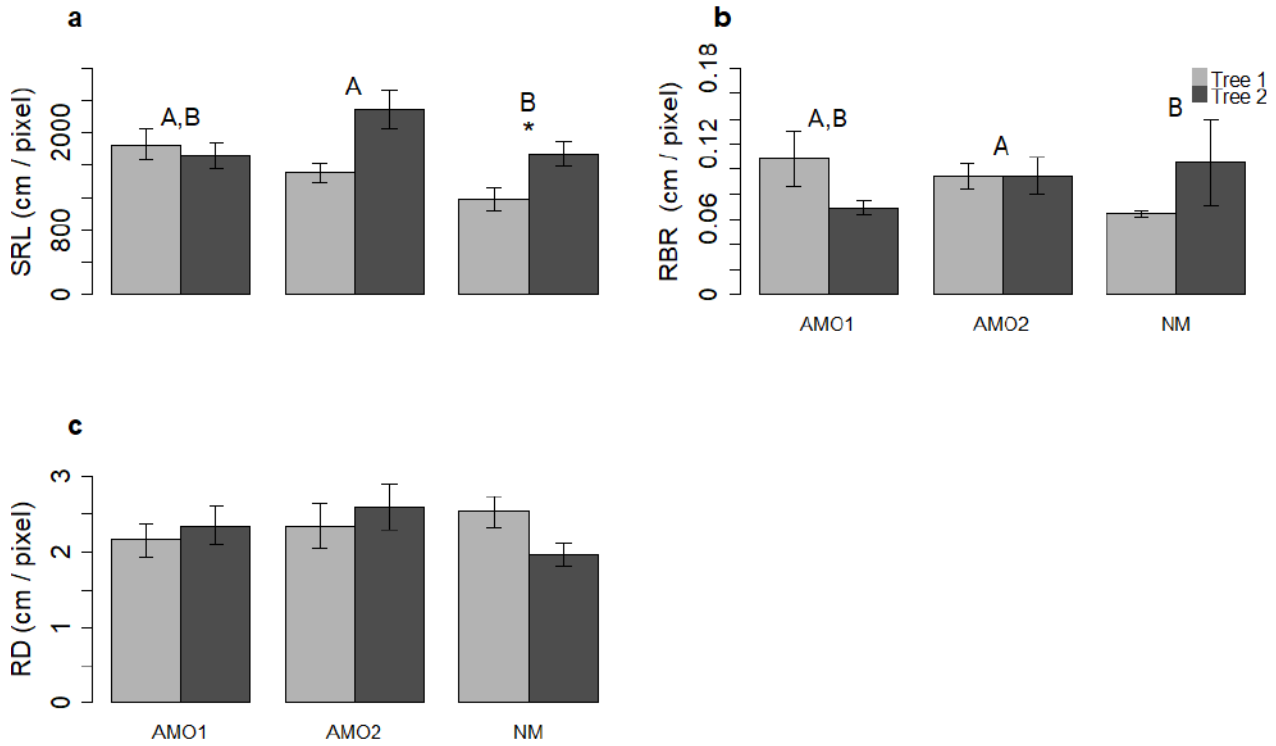

Fig. 3 Interactive effects of origin of seed and AM inoculation on (a) specific root length, SRL, (b) root branching ratio, RBR, and (c) root diameter, RD in *Persea americana* plants that grew with seed from tree 1 (light grey bars) or tree 2 (grey bars) inoculated with AM fungi from the O1 (AMO1), O2 (AMO2) and a control (NM). Different letters above the bars indicate statistically significant differences among AM treatment according to *t*-test contrast *P* (<0.05), and asterisks indicate statistically significant differences between plants from tree 1 and 2 within the same inoculum treatment. Mean ± standard errors were given without data transformation.

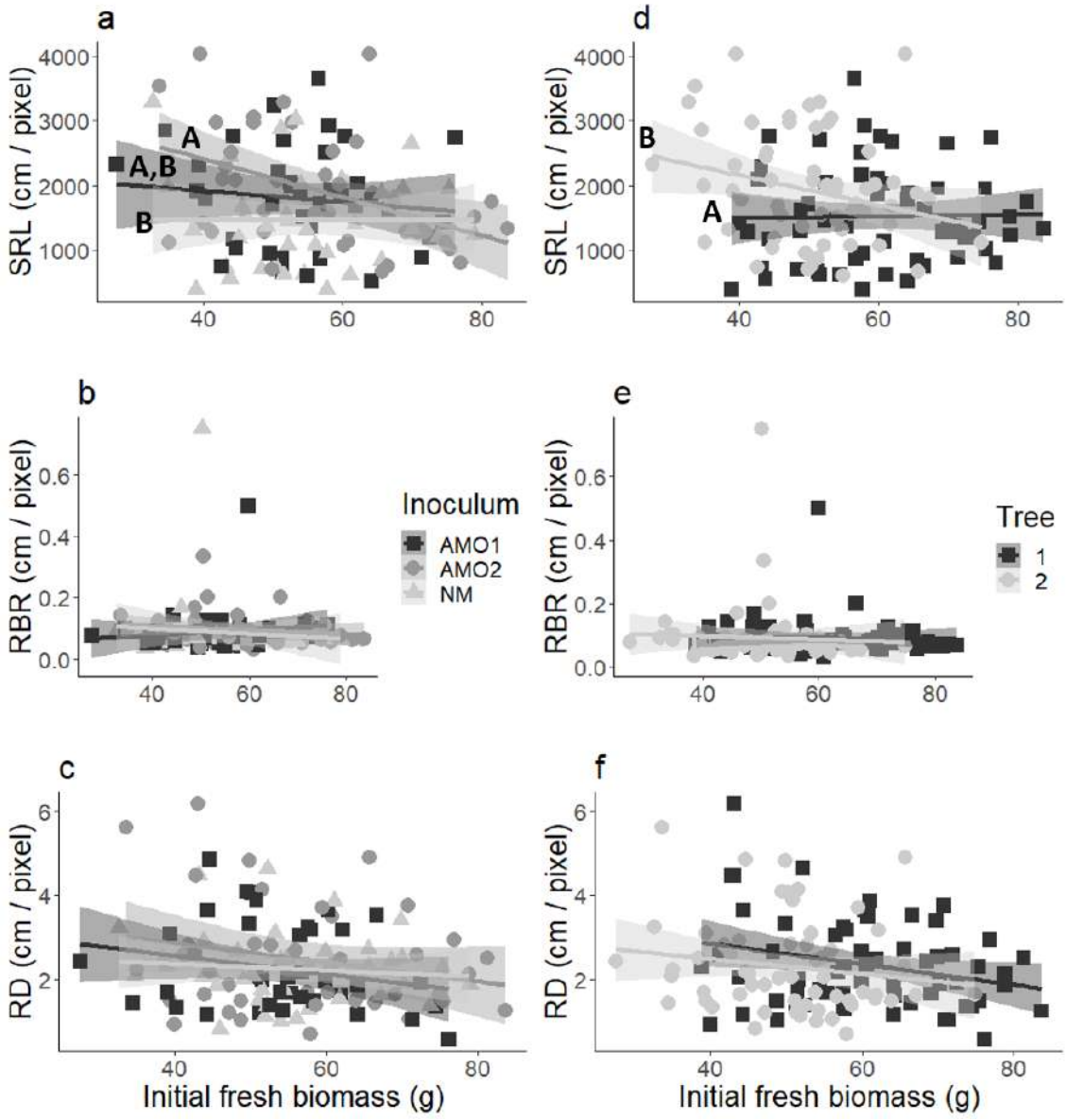

Fig. 4 Interactive effects of the AM inoculation and the initial fresh biomass (covariate) on (a) specific root length, SRL, (b) root branching ratio, RBR, and (c) root diameter, RD, in *Persea americana* plants inoculated with AM fungi from the O1 (AMO1, black square), O2 (AMO2, grey circle) and a control (NM, light grey triangle). The interactive effects of the seed origin and the initial fresh biomass (covariate) on (d) specific root length, (e) root branching ratio and (f) root diameter in *Persea americana* plants that grew with seed from tree 1 (black squares) or tree 2 (grey circles). The respective lines represent the regression lines with their confidence intervals (shaded). Different letters above the lines indicate statistically significant differences among AM inoculum or origin of seed respectively according to *lsmeans* contrast *P* (<0.05).

Plant biomass

The origin of the seed significantly affected the RGR and BB (Table 1). The plants from T2 had a higher RGR (mean ± standard error, 0.007 ± 0.0001 g g-1 day-1) compared with the plants from T1 (RGR: 0.006 ± 0.0002 g g-1 day-1, Fig. S1a). In contrast, plants from T1 had higher BB (14.812 ± 0.581 g) compared with the seeds from T2 (13.982 ± 0.455 g, Fig. S1c).

The origin of soil significantly also affected AB and R/S (Table 1). The plants growing with soil from O2 had higher AB (15.191 ± 0.460 g) compared with the plants from O1 (13.160 ± 0.558 g). In contrast, plants growing with soil from O2 had smaller R/S (1.075 ± 0.143) compared with the plants from O1 (1.237 ± 0.085).

AM inoculum only significantly affected the variable AB (Table 1). The plants growing with the AMO1 (14.046 ± 0.535 g) and AMO2 (15.363 ± 0.510 g) treatments had higher AB (without differences between them, AMO1-AMO2 p= 0.332) compared with the plants in the NM treatment (13.118 ± 0.809 g). The NM treatment did not differ with the AMO1 treatment (AMO1-NM p= 0.644). The plants growing with AMO1 inoculum (0.006 ± 0.0002 g g-1 day-1) and AMO2 (0.006 ± 0.0001 g g-1 day-1) tended to have higher RGR (marginally non-significant differences, Table 2), compared with the treatment without mycorrhizae (0.006 ± 0.0002 g g-1 day-1). In contrast, plants growing in the NM treatment tended to have higher R/S ratio (1.444 ± 0.239 g, marginally non-significant differences, Table 1) compared to AMO1 (1.045 ± 0.044 g) and AMO2 (0.979 ± 0.042 g) treatments.

The interaction between the soil origin and the seed origin affected the AB and R/S (Table 1). The AB showed significant differences with the origin of seed in O1 (Fig. S1b). Avocado plants from T2 developed higher AB compared to those from T1 (Fig. S1b). In general, avocado plants growing in soil from O2 developed higher AB (Fig. S1b). On the other hand, plants growing in the O1 had greater R/S ratio compared with the plants from O2 (Fig. S1d). We did not find significant differences between the interaction soil origin and seed origin explaining the RGR and the BB (Fig. S1a and S1c).

The interaction between AM inoculum and origin of seeds affected the above- and belowground biomasses, and R/S ratio. The plants growing without mycorrhizas and from T1 had significantly lower AB than plants from T2 (Fig. S2b), without differences between plants from T1 and T2 growing with mycorrhizas (Fig. S2b). The plants from T1 allocated lower BB in the AMO1 than plants from T2, and there were not significant differences between plants from T1 and T2 growing with AMO2 and NM (Fig. S2c). On the other hand, in the R/S ratio, the plants from T1 had greater R/S ratio than plants from T2 growing in the NM treatment (Fig. S2d).

Finally, the covariate with the AM inoculum affected the RGR, AB and R/S ratio (Table 1). The AM treatments produced greater RGR in smaller plants; in contrast, the NM treatment produced greater RGR in plants with greater IFB (Fig. 5a). The plants in the AM treatments constantly produced greater AB compared with NM treatment, and larger plants had greater aboveground biomass (Fig. 5b) independently of AM inoculum. The belowground increased with the weight of the plants, but there were not significant differences between AM treatments (Fig. 5c). In smallest avocado plants growing in the NM treatment, the R/S ratio was greater than AM treatments and independently of their weight (Fig. 5d).

Fungal measurements

The fungal parameters measured showed significant differences for the origin of seed and soil (Table 3). The plants from T2 had greater colonization by hypha (Fig. S3a, 80.064 ± 2.408%) and vesicles (Fig. S3b, 67.132 ± 3.040%) compared with those from T1 (hypha: 63.832 ± 4.345%, vesicles: 43.062 ± 4.201%). The plants growing in the soil from O1 had greater colonization by hypha (82.765 ± 2.704%), vesicles (63.418 ± 4.058%) and arbuscules (19.836 ± 2.653%) compared with those growing with the inoculum from O2 (hyphae: 61.132 ± 3.841%, vesicles: 46.776 ± 3.781% and arbuscules: 8.343 ± 1.860%). At the end of the experiment, all NM plants remained non-mycorrhizal.

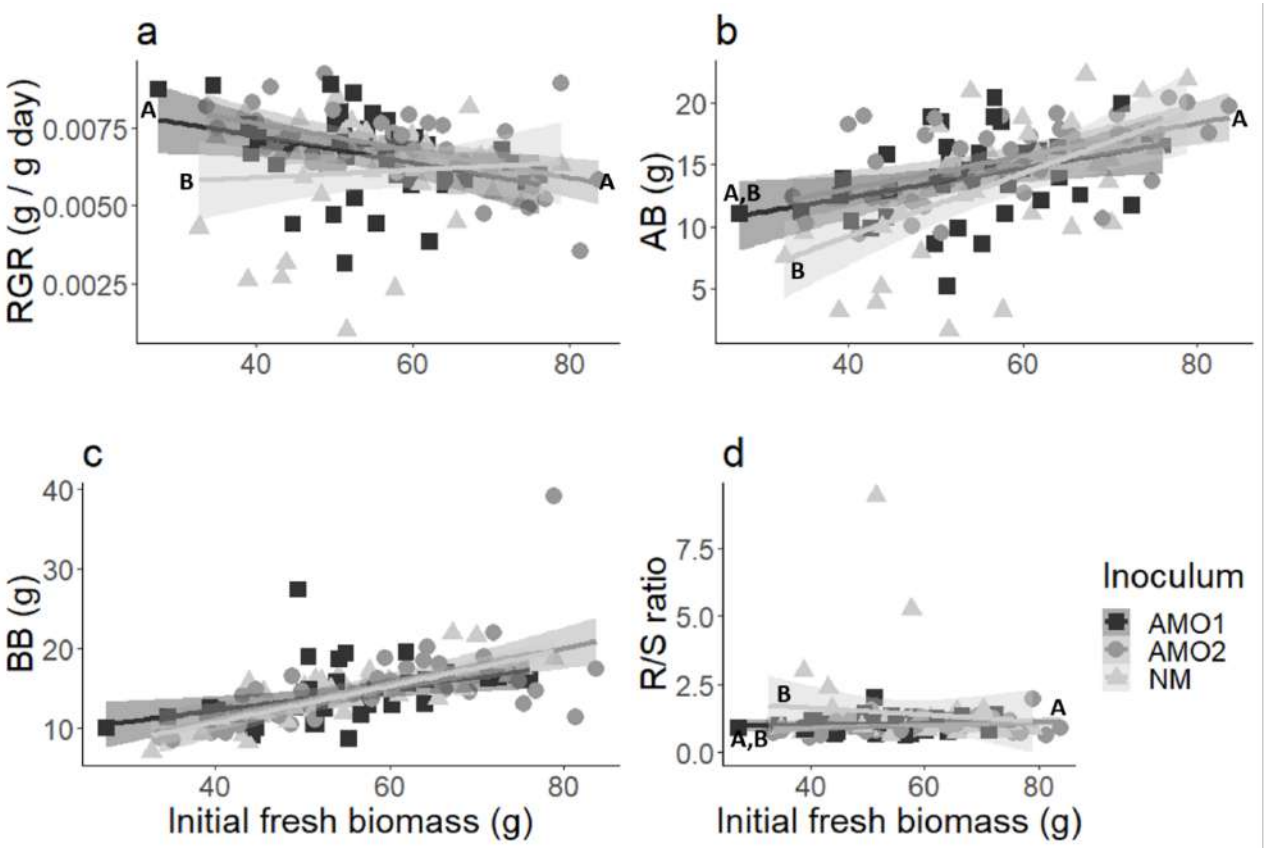

Fig. 5 Interactive effects of the AM inoculation and the initial fresh biomass (covariate) on (a) relative growth rate, RGR, (b) aboveground dry biomass, AB, (c) belowground dry biomass, BB, and (d) root/shoot ratio, R/S, in *Persea americana* plants inoculated with AM fungi from the O1 (AMO1, black square), O2 (AMO2, grey circle) and a control (NM, light grey triangle). The respective lines represent the regression lines with their confidence intervals (shaded). Different letters above the lines indicate statistically significant differences among AM inoculum according to *lsmeans* contrast *P* (<0.05).

Table 3 Summary of the statistical results of ANCOVA models for the fungal structures analyzed in *Persea americana*.

Source of variation	df	Hypha (%) F	Hypha (%) P	Vesicle (%) F	Vesicle (%) P	Arbuscule (%) F	Arbuscule (%) P
T	1	15.189	**<0.001**	28.694	**<0.001**	1.759	0.189
O	1	26.981	**<0.001**	16.537	**<0.001**	14.886	**<0.001**
In	1	1.008	0.318	0.121	0.729	1.384	0.243
IFB	1	0.099	0.753	1.516	0.222	4.577	**0.036**
T:O	1	0.535	0.466	0.300	0.585	0.398	0.530
T:In	1	8.146	**0.005**	6.796	**0.011**	6.829	**0.011**
O:In	1	4.314	**0.041**	4.505	**0.037**	6.353	**0.014**
T:IFB	1	0.033	0.854	1.000	0.320	0.292	0.590
O:IFB	1	0.128	0.720	0.517	0.474	0.318	0.574
In:IFB	1	1.240	0.269	0.582	0.448	1.339	0.251
T:O:In	1	0.008	0.927	0.334	0.564	0.337	0.563
T:O:IFB	1	1.524	0.221	8.49	**0.004**	1.720	0.194
Residuals	67						

T: origin of seeds (T1, T2); O: origin of soil (O1, O2); In: origin of AM inoculum (AMO1, AMO2); IFB: initial fresh biomass.

There were statistically significant differences in the interaction between AM inoculum and origin of seeds explaining the percentages of hyphae, vesicles and arbuscules (Fig. S3). The percentages of hypha, vesicle and arbuscules were greater in the roots from T2 and growing in the AMO2 (Figs. S3a, S3b and S3c).

An important interaction occurred among AM inoculum and origin of soil explaining the percentages of fungal structures (Table 3). The plants growing in the soil from O1 and with AM inoculum isolated from the same orchard had greater percentage of AM colonization by hyphae, vesicles and arbuscules (Figs. 6a, 6b and 6c). In contrast, there were not statistically significant differences for the plants inoculated with mycorrhizas from O2. However, the AM colonization with inoculum from O1, tended to decrease in presence of soil from O2, and the AM colonization tended to increase with the presence of AM inoculum from AMO2 (Fig. 6).

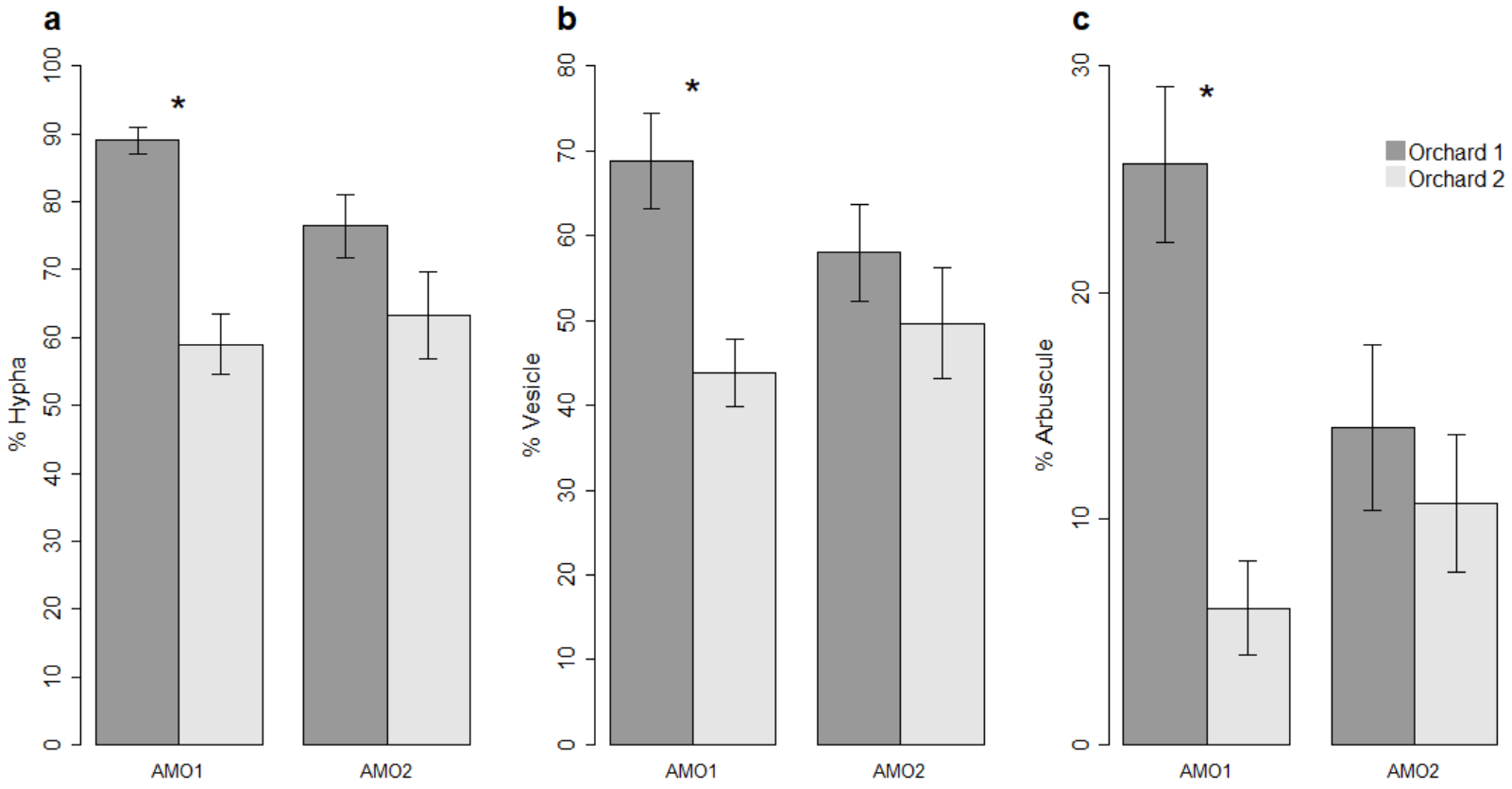

Fig. 6 Interactive effects of the soil origin and AM inoculation on percentage of (a) hypha, (b) vesicle and (c) arbuscules in *Persea americana* plants that grew with soil from orchard 1 (grey bars) or orchard 2 (light grey bars) and inoculated with AM fungi from O1 (AMO1) and O2 (AMO2). Asterisks indicate statistically significant differences between plants growing in orchard 1 and 2 within the same inoculum treatment. Mean ± standard errors were given without data transformation.

Discussion

In this study, we investigated whether the origin of seed, soil and AM propagules influence the effectivity and infectivity of avocado plants. The soil and AM propagules were obtained in two orchards with contrasting soil agricultural management. Our initial hypotheses were partially support, given that we found little evidence that soil and AM origin affected negatively the performance of plants in terms of chlorophyll fluorescence, root traits, and biomass, but there was evidence that the infectivity is greater in plants growing with soil and AM inoculum from the orchard without soil agricultural management.

Chlorophyll fluorescence

The fluorescence emission of photosynthetic systems *in vivo* conditions changes continuously following their adaptation to the environment (González et al. 2008), and some physical or chemical environmental factors such as changes in light intensity, presence of heavy metals, herbicides, availability of mineral nutrients, temperature among others, could decrease F_v/F_m, indicating plant stress (e.g., Zhu et al. 2010, 2011). In the present study, the F_v/F_m ratios fluctuated around values close to 0.8, suggesting that avocado plants were not stressed. Likely due to nursery garden conditions than treatments (origin of seeds, origin of soil and AM inoculum), given that we did not control the temperature and humidity. However, the initial biomass of plants, along with the origin of the seeds from tree 1 and tree 2 affected the F_0 and F_v/F_m. The F_v/F_m from weightier plants (T1) was greater than lower plants. The values of F_v/F_m may be due to increase in F_0 or decrease in F_m, and under stress conditions an increase in F_0 or a decrease in F_m reflects the destruction and loss of PSII reaction center or disruption of electron transport for excitation of reaction centers (Baker 2008). In our study F_m was lower in O2, it has been suggested that the deficiency of nutrients such as P, Ca, Mg and Zn have relationship with a decrease in F_m negatively impacting the efficiency of electron transport in photosystem II (Foyer y Spencer 1986; Smethurst et

al. 2005; Wu et al. 2006; Liu et al. 2009; Kalaji et al. 2016). This find is consistent with the values of F_v/F_m in the O2, which tended to be lower than O1.

In our study, F_0 was greater in smaller plants and this is consistent with the lower values of F_v/F_m for smaller plants. This suggest that plants with lower initial fresh biomass were stressed. Ramírez (2016) observed that seedlings from lower biomass seeds have a reduced formation of organs such as leaves and roots, which translates into a lower capacity to perform the metabolic processes essential for their proper development. Given that chlorophyll fluorescence is related with leaf traits (e.g., leaf thickness, photosynthetic capacity per area) is probably that the size and number of leaves in our experiment influenced the F_v/F_m ratio in smaller plants.

The efficient performance of photosystem II in several species growing with AM fungi under stress conditions has been shown (e.g., Zhu et al. 2011, Yooyongwech et al. 2013, Shamshiri and Fattahi 2016). For instance, Zhu et al. (2011), showed that AM fungi protected *Zea mays* plants against high temperature through ameliorating photosynthetic performance. Yooyongwech et al. (2013), noticed values of *Fv/Fm* close to 0.8 in *Macadamia tetraphylla* L. plants with AM inoculum than non-mycorrhizal plants when exposed to water deficit. Shamshiri and Fattahi (2016), showed in *Pistacia vera* inoculated with AM fungi an increase in photosynthesis performance under salt stress as well as under normal conditions. In avocado plants, Castro-Alvarado et al. (2013) quantified the chlorophyll concentration in leaves of mexican landrace avocado rootstocks with and without AM fungi. They showed that avocado plants with AM inoculum had 20% higher chlorophyll concentration compared with the non-mycorrhiza inoculum, suggesting that these fungi contribute a higher photosynthetic capacity. However, in this study the AM inoculation did not affected the chlorophyll fluorescence parameters evaluated. Although the interaction among the origin of seeds, soil and AM inoculum was significant suggesting that the combination of several factors is an important driver of their photosynthetic activity. The chlorophyll fluorescence is a good measurement about the physiological condition of plants and it is used to predict crop yields under various environmental conditions (Goltsev et al. 2016). Nevertheless, to

our knowledge, no studies have been performed in avocado plants despite that Mexico is the largest producer of avocado worldwide.

Root traits

Fine roots, like leaves, are primary resource-acquisition organs. Root traits influencing plant performance and biogeochemical processes that can shift in function from resource absorption to transport (Laliberté 2017). Therefore, the structures and function of the roots are expected to influence how plants respond to colonization by AM fungi (Maherali 2014, Xue-Guang and Ming 2013). Greater specific root length increases absorptive area per unit mass. The avocado plants growing with AM inoculum had lower SRL compared with non-mycorrhizal plants, and the plants from orchard 1 tended to have lower SRL than plants from orchard 2. The same pattern was found for the root branching ratio. This finding is agreeing with several studies suggesting that plants with lower SRL and RBR frequently have a high mycorrhizal dependency (e.g. Maherali 2014, Liu et al. 2015, Vega-Frutis et al. 2015, Laliberté 2017). Although, the concentrations of several soil mineral nutrients, especially P and N from orchard 1, could indicate lower mycorrhizal dependency. It is probable that these mineral nutrients are in a form unavailable to plants.

Salazar (2002), reported that avocado roots lack of radical hairs, and Barea et al. (1997) showed that AM hyphae compensate for the function of the radical hairs at a lower metabolic cost. This is consistent with studies suggesting that the avocado is highly dependent on the AM fungi (Gómez et al. 2012; Rivera et al. 2016). However, the root traits in avocado plants could respond plastically to fungal colonization, soil mineral nutrients or genetic variability between the individuals. In this way, the bigger seeds (from tree 2) and seedlings (initial fresh biomass) produced greater SRL suggesting that root traits depend of environment conditions and of phylogenetic history (Chen et al. 2013, Kong et al. 2014). Ramírez (2016) observed that avocado seeds with intermediate mass (40-50 g) have enough reserves to germinate and once the seed reserves are depleted, take advantage of AM fungi.

To our knowledge, this is the first study analyzing the avocado root traits. However, it is important defining if root parameters influence mycorrhizal effectiveness. This will help to reduce the amounts of fertilizers required to achieve the same yield, and so reduce the negative effects that the agricultural practices have on soil ecosystems and function of these.

Plant biomass

In general, the AM fungi improved the plant biomass parameters evaluated, these finding agree with many papers showing the positive effect of AM fungi on several crops (Naher et al. 2013; Berruti et al. 2016; Rillig et al. 2016; Ryan and Graham 2018, Rillig et al. 2019). However, the outcomes of the AM fungal symbiosis are strongly context dependent, and several biotic factors such as the traits of the roots, weight of seeds, and the identity of AM fungi, along with soil, mineral nutrients modulate the AM fungal effect on the yield of crop plants (Zhang et al. 2018).

In this study, we found that initial weight of seeds and seedlings correlate with resource's allocation to above-, belowground biomasses and RGR. In general, smaller plants allocate lower resources to above- and belowground biomasses, but greater resources to RGR. It has been observed that smaller biomass seeds consume their reserves quickly compared with seeds of greater biomass. Therefore, the seedlings in its initial stages had a develop slow, given that they can obtain nutrients quickly from its reserve tissues (Ramírez 2016). However, the smaller seeds allocated more resources to growth when were inoculated with AM fungi, and this is consistent with another studies (e.g., Varga et al. 2013). At the end of experiment, the biomasses parameters in the avocado plants were greater in AM plants than non-mycorrhizal plants.

Regarding to abiotic factors, soil mineral nutrients and management of the agricultural system influences the AM fungal effect on their host. In our study, the orchard 2 received poultry manure and chemical fertilizer. The belowground biomass was similar between soil origin and mycorrhizal and non-mycorrhizal plants. Given that

the fertilization increases available soil mineral nutrients the cost of allocate resources to roots, likely, is lower than AM fungi (Johnson 2010), and this is consistent with the lower colonization quantify in these plants. Increased P concentration in soil solution decreases mycorrhizal colonization, this could explain because the avocado plants growing with AM from O2 had lower mycorrhizal colonization than AM plants from O1, but similar root biomass. Regarding to aboveground, the plants growing with AM propagules allocated more resources to aboveground biomass than non-mycorrhizal plants, and this allocation was regardless of AM origin. Our findings agree with several studies in avocado plants, for instance, Castro-Alvarado et al. (2013) showed that inoculated plants had greater height (around of 54% more) than non-mycorrhizal plants, and Carreón et al. (2014) observed that *Acaulospora delicate* increases plant height than another AM species. AM genera like *Glomus, Acaulospora and Scutellospora* have shown positive effects on the growth and biomass of young avocado plants (Silveira et al. 2003, Montañez-Orozco 2009). These studies could suggest that avocado plants are dependent of AM fungi as show our results of lower SRL and RBR. Additionally, the plants growing with soil from orchard 2 (with soil nutrition management) had greater aboveground biomass, likely due to the availability of mineral nutrients. The use of fertilizers could generate competition by resources between the plant and the microorganism present in the soil. In this sense, Johnson (2010) suggested that the plants could allocate more resources to growth and/or reproduction, and lower resources to roots and AM fungi (Johnson 2010, Lauriano-Barajas 2018). This could explain because the plants growing in the soil added with chemical nutrition (O2) had higher AB and lower R/S ratio compared with avocado plants growing in soil without nutritional management (O1).

Fungal measurements

The plants growing in the soil from orchard 1 (without nutrient management) had greater root colonization by hyphae, vesicles and arbuscules, likely because this orchard did not receive chemical fertilizers compared with the O2. This finding is

agreeing with many studies suggesting that AM symbioses depends on the stoichiometry, mainly, of available N and P, i.e., low available mineral nutrients is related with higher mycorrhizal colonization (Johnson 2010; Smith and Smith 2011).

Our soil analyses showed that orchard 1 had greater concentration of P and N, along with other nutrients. It is probable that these nutrients are in unavailable forms for the plants, compared with the orchard 2 that received chemical nutrients that could be available for the plants. In addition, it has been observed that a high content of organic matter leads to the microorganisms of the rhizosphere successfully colonize the roots of plants due to the penetrability of the soil (Castellanos et al. 2000). In the analyses of sterilized soil, we observed that the O1 presented a very high content of organic matter than O2. We also observed, although lower, mycorrhizal colonization in the plants growing in the soil from O2.

Previous studies have reported that agricultural practices such as fertilization has a negative effect not only on mycorrhizal colonization, but also on fungal propagules (Wagg et al. 2014; Trejo et al. 2016; Vega-Frutis et al. 2018). Given that there was not growth depression in the plants from O2 compared with the O1, it is probable that the plants could be using both fine roots and AM hyphae to uptake mineral nutrients efficiently (Smith and Smith 2011). Although greenhouse experiments have shown lower percentages of mycorrhizal colonization with increasing soil P, at intermedia soil P levels will reflect relatively root growth, as was observed in our experiment (similar belowground biomass in AM treatments). A study focusing on decreased colonization in tomato (Nagy et al. 2009) included an experiment with intermediate P supply and showed that formation of arbuscules, and amount of P taken up via the AM fugal pathway remained constant per plant, even though mycorrhizal colonization went down.

We observed that plants growing in soil from O1 (without nutrients management) and inoculated with AM propagules from the same orchard had greater percentages of all fungal structures quantify. Johnson et al. (2010) done a reciprocal AM inoculation experiment to test if soil is a key driver of local adaptation in arbuscular mycorrhizal

symbioses, they showed that the plants (*Andropogon gerardii*) are adapt to their local soil and indigenous AM fungal communities. They also observed more percentage of arbuscules (specialized fungal structure for nutrient exchange between fungi and their host plant). In our study, we observed the same pattern that Johnson et al. (2010), and as they pointed up, the soil management conditions driver the symbioses among plants and soil microorganism. Therefore, edaphic origin of AM fungi should be considered in the agricultural management programs to obtain a sustainable agriculture, given that AM fungi is part of most crops.

Finally, several studies in avocado plants both field and greenhouse conditions have shown that avocado plants are mycotrophic. For instance, several studies in avocado have been observed colonization percentages above 75% (Osorio et al. 2012; Carreón-Abud et al. 2013; Castro-Alvarado et al. 2013; Carreón-Abud et al. 2016; Rivera et al. 2016). However, our findings showed that the response variables analyzed, and the biotic (seed genotypes) and abiotic (mineral soil nutrients) factors are important drivers of the outcomes observed in the avocado plants. When considering multiple mycorrhizal-influenced parameters it may also became evident that mycorrhizal may positively influence some plant traits and others negatively. Despite that in this work the diversity of AM fungi present in the orchards was not analyzed, it is important to know the species present, since many of these are determinants in the benefits reflected in the plants and in the soil. In addition, in O2 the fertilizer combination has been decisive so as not to completely affect the beneficial effects of mycorrhizae present in this orchard.

Conclusions

Both the origin of seeds and origin of soil were determinant factors for plant effectiveness (chlorophyll fluorescence, root traits, plant biomass) and infectivity (mycorrhizal colonization), and the AM inoculum of both orchards was infective and effective in avocado plants. However, the origin of the soil and origin of AM inoculum showed a local adaptation.

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Supplementary material

Table S1 Summary of sterilized soil parameters quantified from orchard 1 (O1) and orchard 2 (O2) located in the State Reserve Sierra de San Juan, Nayarit, Mexico.

	Determination	Properties	
		O1	O2
mg kg^{-1} = ppm	P-Bray	52	2
	K	503.1	390
	Ca	1966	544
	Mg	738.8	133.65
	Fe	82.5	77.9
	Zn	2.2	0.4
	Mn	147.4	103.2
	Cu	0.3	1.12
%	OM	6.19	3.25
	TN	0.87	0.66
	pH (1:2 H_2O)	6.31 (mod. acid)	5.21 (mod. acid)

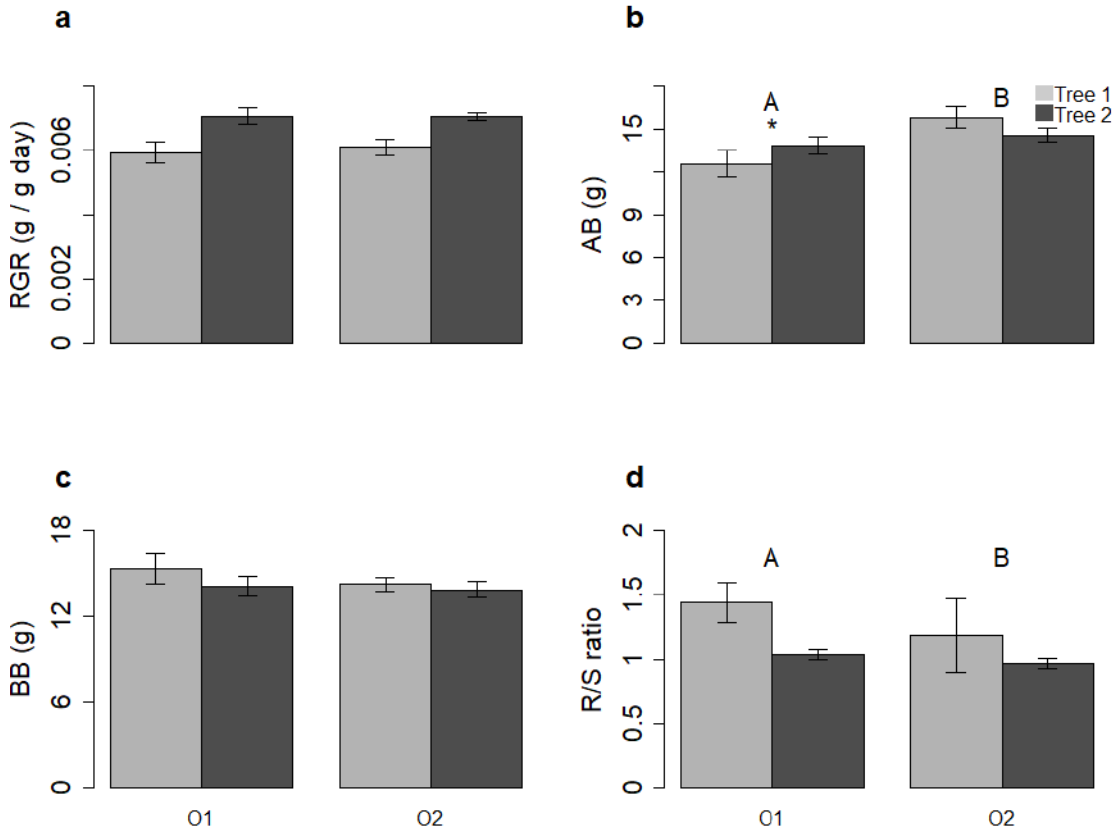

Fig. S1 Interactive effects of the seed origin and the soil origin on (a) relative growth rate, RGR, (b) aboveground dry biomass, AB, (c) belowground dry biomass, BB, and (d) root/shoot ratio, R/S, in *Persea americana* plants that grew with soil from the O1 (grey bars) and O2 (black bars). Different letters above of the bars indicate statistically significant differences among orchard according to *t-test* contrast *P* (<0.05), and asterisks indicate statistically significant differences between plants from seeds of tree 1 and 2 within the same orchard. Mean ± standard errors were given without data transformation.

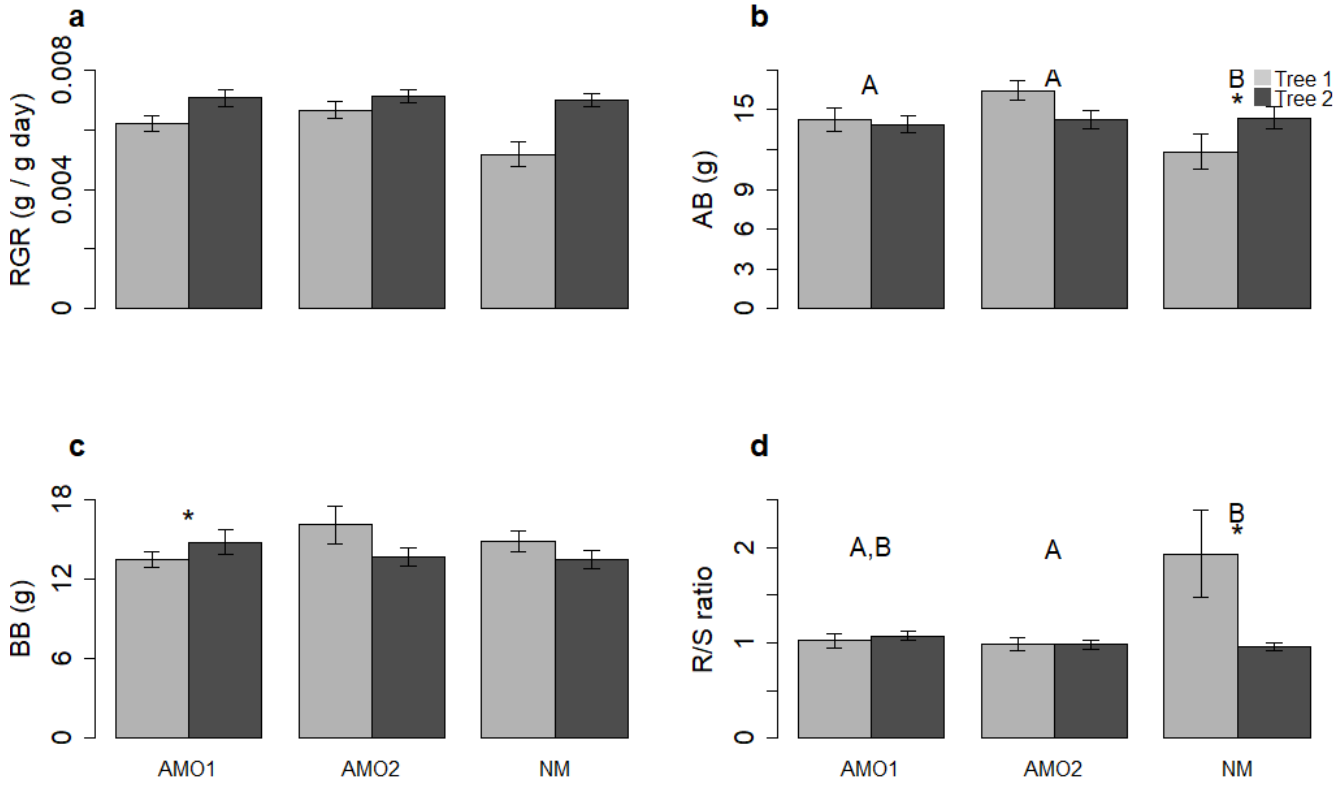

Fig. S2 Interactive effects of the seed origin and AM inoculation on (a) relative growth rate, RGR, (b) aboveground dry biomass, AB, (c) belowground dry biomass, BB, and (d) root/shoot ratio R/S, in *Persea americana* plants that grew with seed of the tree 1 (light grey bars) or tree 2 (grey bars) inoculated with AM fungi from O1 (AMO1), O2 (AMO2) and a control (NM). Different letters above the bars indicate statistically significant differences among AM inoculum according to *t-test* contrast *P* (<0.05), and asterisks indicate statistically significant differences between plants from seeds of tree 1 and 2 within the same inoculum treatment. Mean ± standard errors were given without data transformation.

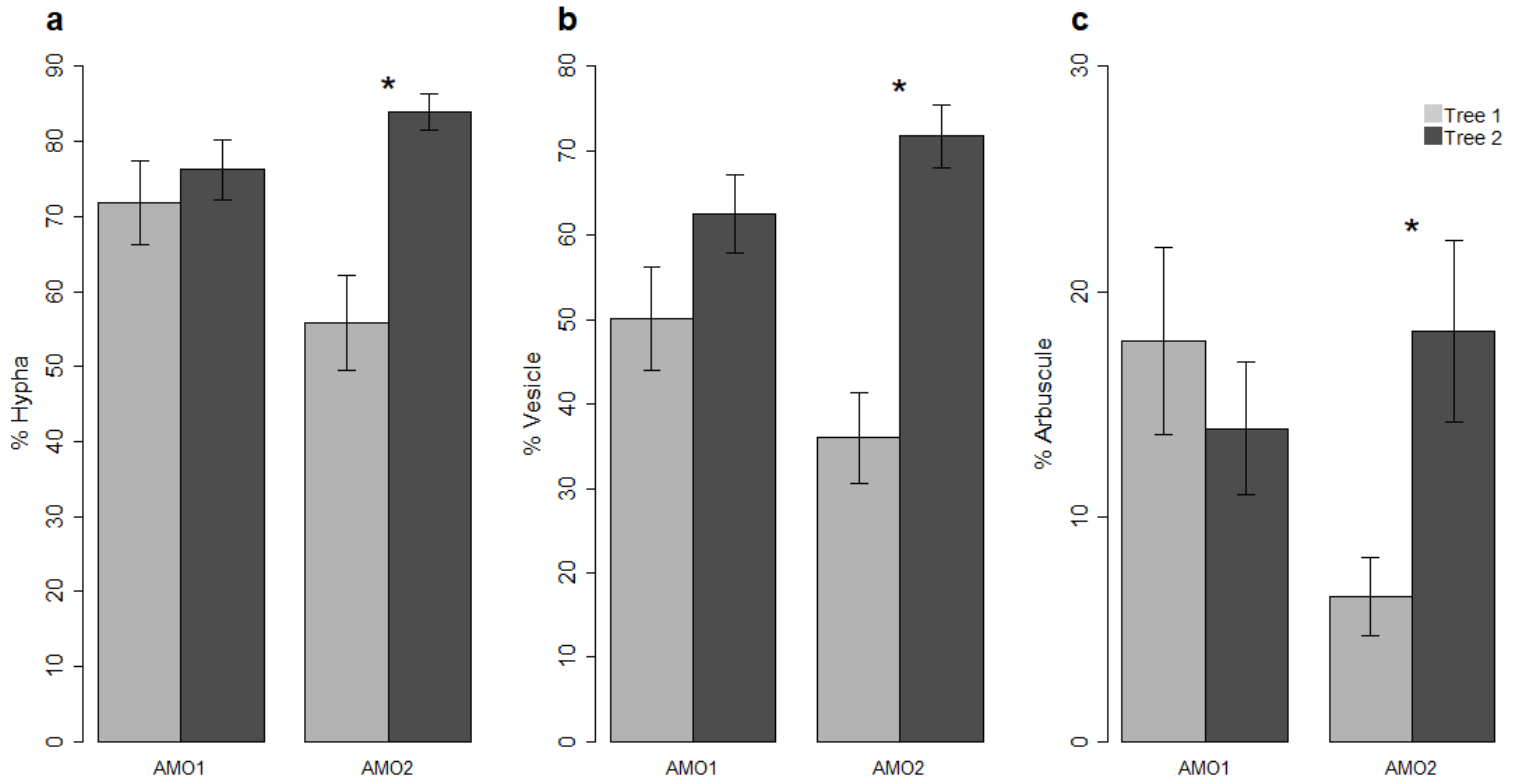

Fig. S3 Interactive effects of the seed origin and AM inoculation on (a) percentage of hypha, (b) percentage of vesicle and (c) percentage of arbuscules in *Persea americana* plants that grew with seed from tree 1 (light grey bars) or tree 2 (grey bars) inoculated with AM fungi from O1 (AMO1) and O2 (AMO2). The asterisks indicate statistically significant differences between plants from seeds of tree 1 and 2 within the same inoculum treatment. Mean ± standard errors were given without data transformation.

CAPÍTULO VI

REFLEXIÓN: LAS MICORRIZAS EN LOS AGROECOSISTEMAS

Durante los últimos años se han publicado varios artículos sobre el papel que juegan los hongos micorrizógenos arbusculares (HMA) en las especies cultivadas, principalmente porque: 1) los HMA se encuentran distribuidos en prácticamente todos los agroecosistemas, con algunas excepciones como especies hortícolas y ornamentales de la familia Brassicaceae, 2) por su papel multifuncional en las plantas, es decir, mejoran el estatus nutricional de estas, funcionan como bioprotectores contra factores bióticos (herbívoros y patógenos) y abióticos (salinidad, sequía, y metales pesados), además de ser importantes en la estabilidad y formación de los suelos, 3) porque las prácticas agrícolas, tales como la labranza, fungicidas, herbicidas y los fertilizantes tienen un efecto negativo sobre la diversidad y abundancia de estos hongos, potencialmente afectando su función (infectividad y efectividad), y 4) porque los HMA pueden ser usados como bioinoculantes (Rillig et al. 2016; Rillig et al. 2019).

Así, es importante entender la contribución relativa de los HMA en la sustentabilidad de los agroecosistemas, estudiar y conocer que parámetros influyen la efectividad de los hongos, y las variables de respuesta adecuadas para documentar los efectos de las micorrizas sobre sus plantas hospederas.

Cuando se consideran múltiples parámetros, algunas características de las plantas muestran un efecto positivo (ej. mayor crecimiento), pero en otras parece no ser benéfica o negativa (ej. número de frutos). No obstante, los HMA se encuentran interactuando con varios organismos del suelo, además de sus plantas hospederas y responden dinámicamente al manejo de los agroecosistemas. Sin embargo, el que los productores incluyan los HMA en sus cultivos no es tarea fácil, dado que implica un cambio radical de la manera en que se ha maneja la agricultura, pero este estudio pretende dar a conocer la importancia de los HMA sobre el mejoramiento de los servicios que ofrecen los HMA y aumentar la producción de la agricultura sustentable (Gianinazzi et al. 2010; Smith y Smith 2011; Berruti et al. 2016; Rillig et al. 2016; Rillig et al. 2019; Lauriano-Barajas y Vega-Frutis 2018; Vega-Frutis et al. 2018).

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CONCLUSIONES GENERALES

- En campo, los porcentajes de colonización por HMA estuvieron relacionados con los nutrientes minerales del suelo, y fueron independientes del tamaño del árbol.
- En vívero, la combinación de los factores 1) origen de la semilla, 2) inóculo micorrícico, y 3) origen del suelo, fueron determinantes en la actividad fotosintética.
- Las plantas con menor longitud de raíz específica y baja proporción de ramificación de la raíz fueron altamente dependientes de las micorrizas, esto se relaciona con el alto contenido de materia orgánica, comparado con la parcela que recibe manejo nutricional.
- La biomasa de las semillas y plántulas fue importante en los recursos asignados a la biomasa aérea y subterránea.
- La combinación de nutrientes químicos y orgánicos aplicados a la parcela 2, ha permitido que la simbiosis micorrícica no se vea atenuada del todo.
- Aunque los HMA no son hospederos específicos, si existe un grado de preferencia y co-adaptación. Las plantas creciendo con suelo de la parcela sin manejo nutricional e inoculadas con los propágulos fúngicos extraídos de la misma parcela presentaron mayor porcentaje de colonización micorrícica.
- Este es el primer estudio para el estado de Nayarit dónde se reporta la infectividad y efectividad de los HMA en aguacate.