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"La importancia de la fertilización orgánica en las interacciones bióticas entre hongos micorrízicos arbusculares (HMA), insectos herbívoros y cultivares de maíz"

TESIS

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M.C. Ricardo Aguilar Aguilar

Directores de tesis:

Dra. Yazmín Carreón Abud y Dr. John Larsen

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RESUMEN

Las interacciones entre las raíces de las plantas y su microbiota asociada afectan el crecimiento y la nutrición de las plantas, por lo tanto, los artrópodos de niveles tróficos superiores como herbívoros, parasitoides y polinizadores también son afectados indirectamente.

Los hongos micorrízicos arbusculares (HMA) y otros microorganismos asociados a las raíces afectan en diferentes aspectos a los insectos herbívoros, como en su rendimiento respecto a su nivel de herbivoría y parámetros de su ciclo de vida, incluyendo el crecimiento, la sobrevivencia, la fecundidad y la densidad poblacional. Por lo tanto, para llevar a cabo un manejo agroecológico de plagas adecuado de los cultivos, es importante profundizar el conocimiento sobre cómo las prácticas agrícolas afectan tales interacciones entre plantas, microorganismos asociados con las raíces y los insectos herbívoros foliares.

Este estudio planteó la hipótesis de que una mayor cantidad de materia orgánica en el suelo contiene una mayor proporción de microorganismos benéficos, que proporcionan más nutrientes a la planta y, a su vez, le otorga una mayor tolerancia contra los insectos herbívoros. Sobre esta base, se propusieron los siguientes objetivos: 1) evaluar el efecto de la fertilización orgánica y mineral en el rendimiento de diferentes cultivares de maíz y los microorganismos asociados a sus raíces, y 2) determinar cómo la fertilización orgánica y mineral afectan las interacciones bióticas entre hongos micorrízicos arbusculares (HMA), cultivares de maíz e insectos herbívoros.

Se llevaron a cabo cuatro experimentos para cumplir con los objetivos descritos anteriormente. El primero de ellos evaluó el rendimiento de la planta y los microorganismos asociados en cuatro cultivares de maíz (2 nativos y 2 híbridos) y seis fertilizantes orgánicos (abono verde, estiércol y composta) como los dos factores principales. En el segundo y tercer experimento, se evaluó la influencia de la

fertilización orgánica y mineral en la herbivoría de larvas de *Spodoptera frugiperda* en dos cultivares de maíz (híbrido y nativo). En el cuarto experimento, se evaluó la influencia de tres aislados puros de HMA en la herbivoría de larvas de *Spodoptera exigua* en dos cultivares de maíz (híbridos y nativos).

Los resultados más relevantes del experimento uno, mostraron que la fertilización orgánica y mineral afectaron positivamente a los cultivares de maíz, obteniendo el mayor crecimiento de plantas de maíz con fertilización mineral, seguida de la fertilización orgánica. El estiércol de res, la composta y el lombricomposta aumentaron el crecimiento de las plantas, mientras que los tres tipos de abono verde no tuvieron ningún efecto sobre el crecimiento del maíz. La colonización de raíces con HMA se redujo con el estiércol de res y los abonos verdes janamargo y canola. La infección por los patógenos de raíces *Pythium* y *Polymyxa* se redujo con todos los fertilizantes orgánicos, mientras que la infección con el patógeno *Microdochium* aumentó con la mayoría de los fertilizantes orgánicos. En los experimentos dos y tres, la herbivoría de *S. frugiperda* en el maíz no se vio afectada por ninguno de los factores estudiados, como el tipo de fertilización y la esterilización del suelo. Además, en el caso de la herbivoría total en la parte de la hoja por *S. frugiperda*, ni el crecimiento de la raíz ni la colonización de las raíces por HMA, se vieron afectados, mientras que en el caso de la herbivoría moderada, hubo un ligero aumento en la colonización de las raíces con HMA. En el experimento cuatro, se observó una disminución en la relación C/N en el tejido foliar de maíz inducido por las tres especies de HMA examinadas, lo que resultó en una mayor biomasa de larvas de *S. exigua*.

A partir de estos resultados, se concluye que el tipo de cultivar de maíz y la aplicación de fertilizantes orgánicos afectan la abundancia de las poblaciones nativas de HMA en el campo y los patógenos de las raíces en las plantas de maíz, además, la herbivoría de *S. frugiperda* en el maíz no se vio afectada por cualquiera de los factores estudiados, como el tipo de fertilización y esterilización del suelo. También

se concluye que los HMA mejoran la calidad nutricional del maíz para *Spodoptera exigua*, independientemente de la fertilización con fósforo.

Palabras Clave:

Fertilización orgánica y mineral; hongos micorrízicos arbusculares (HMA); microorganismos patógenos; *Spodoptera frugiperda*; *Spodoptera exigua*; cultivares de maíz.

ABSTRACT

Interactions between plant roots and their associated microbiota affect plant growth and nutrition and thereby indirectly also aboveground higher trophic level arthropods like herbivores, parasitoids and pollinators.

Arbuscular mycorrhizal fungi (AMF) and other root associated microorganisms affect different aspects of insect herbivores such as their performance in terms of level of herbivory and lifecycle parameters including growth, survival, fecundity and population density. Hence, to employ an agroecological crop pest management it is important to improve the knowledge about how agricultural practice affect such interactions between plants, root associated microorganisms and foliar insect herbivores.

This study hypothesized that a greater amount of organic matter in the soil contains a higher proportion of beneficial microorganisms, which provide more nutrients to the plant and this, in turn, gives it greater tolerance against insect herbivory. On this basis, the following objectives were proposed: 1) to evaluate the effect of organic and mineral fertilization on the performance of different maize cultivars and the microorganisms associated to their roots, and 2) to determine how organic and mineral fertilization affect the biotic interactions between arbuscular mycorrhizal fungi (AMF), maize cultivars and herbivorous insects.

Four experiments were carried out to comply with the objectives described above. The first evaluated the plant performance and the associated microorganisms in 4 maize cultivars (2 land races and 2 hybrids) and six organic fertilizers (green manure cow manure, and compost) as the two main factors. In the second and third experiment, the influence of organic and mineral fertilization on herbivory by *Spodoptera frugiperda* larvae on two maize cultivars (hybrid and land race) was evaluated. In the fourth experiment, the role of three pure AMF isolates was evaluated in the herbivory by larvae of *Spodoptera exigua* in two maize cultivars (hybrid and land race).

The main results from experiment one, showed that the organic and mineral fertilization positively affected the maize cultivars, obtaining the highest growth of maize plants with mineral fertilization, followed by organic fertilization. Cow manure, compost and vermicompost increased plant growth, while the three types of green manure had no effect on maize growth. . Root colonization with AMF was reduced by cow manure and the green manure fertilizers with vetch and rape. The infection by the root pathogens *Pythium* and *Polymyxa* was reduced by all the organic fertilizers, while the infection with the pathogen *Microdochium* increased with the majority of the organic fertilizers. In experiments two and three, the herbivory by *S. frugiperda* in maize was not affected by any of the factors studied, such as the type of fertilization and soil sterilization. In addition, in the case of total herbivory of the leaf part by *S. frugiperda*, both root growth and colonization of roots by AMF, were not affected, while in the case of moderate herbivory, there was a slight increase in d AMF root colonization. In experiment four a decrease in the C/N ratio was observed in the foliar maize tissue induced by all three AMF species examined, which resulted in a higher biomass of *S. exigua* larvae.

From these results it is concluded that the type of maize cultivar and the application of organic fertilizers affect the abundance of the native AMF field populations and the root pathogens in maize plants, in addition, the herbivory by *S. frugiperda* in maize was not affected by any of the factors studied, such as the type of fertilization and soil sterilization. It is also concluded that AMF improve the nutritional quality of maize by *Spodoptera exigua*, independently of the fertilization with phosphorus.

Keywords:

Organic and mineral fertilization; Arbuscular Mycorrhizal Fungi (AMF); pathogenic microorganisms; *Spodoptera frugiperda*; *Spodoptera exigua*; maize cultivars.

CAPÍTULO 1. INTRODUCCIÓN GENERAL

1.1 Introducción

El maíz (*Zea mays* L.) es uno de los cereales de mayor producción en el mundo, siendo para México un cultivo de gran importancia, ya que cerca del 60% de la producción nacional se destina al consumo humano. Más de la mitad de la superficie sembrada es de temporal, realizada por pequeños productores, con cultivares nativos y un manejo de agricultura tradicional de autoconsumo. Esta agricultura de subsistencia obtiene bajos rendimientos debido a que los agricultores no pueden solventar los gastos necesarios para llevar a cabo un control de plagas y enfermedades adecuado (Kato et al. 2009. Massieu y Lechuga 2002).

Una de las principales limitantes de la producción de maíz en nuestro país son los insectos plaga, para combatir a estos insectos, los agricultores utilizan principalmente plaguicidas químico-sintéticos, que debido a su uso excesivo, contaminan agua, suelo y aire, provocando daños al ambiente (Bahena y Velázquez 2012, Lesur 2005, Rodríguez y De León 2008).

Ante esta situación, es necesario buscar modelos agrícolas alternativos que sean sostenibles a mediano y largo plazo. Las principales prácticas agrícolas que se llevan a cabo para lograr una agricultura sostenible se dividen en dos grandes rubros, por una parte, para el mejorar la fertilidad del suelo se utiliza la rotación de cultivos, el uso de biofertilizantes, los cultivos de cobertura, los abonos verdes y el uso de compostas, por otra parte, para mejorar la regulación de plagas se utiliza la diversidad de cultivos, las prácticas culturales, el control biológico y la modificación del hábitat (Altieri et al. 2012).

Como se menciona, los dos pilares más importantes para una agricultura sostenible son el mejoramiento de la fertilidad del suelo, llamado también Manejo Integrado de la Fertilidad del Suelo (MIFS) y el mejoramiento de la regulación de plagas también llamado Manejo Integrado de Plagas (MIP), debido a que muchas prácticas de manejo de suelo influyen en el manejo de plagas, para evitar confusiones o enfoques reduccionistas, se ha decidido agrupar estos dos pilares en el Manejo Agroecológico de Plagas y Enfermedades (MAPE) (Altieri et al. 2012).

Para llevar a cabo un manejo agroecológico de plagas y enfermedades efectivo, es necesario conocer con más detalle los componentes arriba-abajo del suelo que están involucrados, ante esto, para apoyar la búsqueda de nuevos conocimientos que nos ayuden a comprender estas interacciones, en este trabajo de investigación los experimentos se enfocaron en los siguientes temas principales:

a) Los efectos de los diferentes tipos de fertilización orgánica en el desempeño del maíz y de sus microorganismos asociados.

b) Los efectos de la de fertilización orgánica y mineral en las interacciones bióticas entre el maíz, los hongos micorrízicos arbusculares (HMA) y el gusano cogollero (*S. frugiperda*).

c) Los efectos de los diferentes tipos de hongos micorrízicos arbusculares (HMA) en las interacciones bióticas entre el maíz y el gusano soldado (*S. exigua*).

1.2 El cultivo del maíz

El maíz (*Zea mays* L.), además de ser el cereal de mayor producción en el mundo, es un cultivo de gran importancia para México, ya que cerca del 60% de la producción nacional, incluyendo el autoconsumo rural, es para consumo humano (Massieu y Lechuga 2002). En 2017, México ocupó el quinto lugar a nivel mundial (2.5% del total), con una producción de 28.7 millones de toneladas (Anónimo 2019a), en ese mismo año, el estado de Michoacán, produjo 1.9 millones de toneladas, ocupando el séptimo lugar a nivel nacional (Anónimo 2019b).

Se considera que en México existen, de acuerdo a diferentes autores, entre 41 y 65 razas o cultivares nativos, los cuales corresponden a más de la mitad de la superficie sembrada de maíz, bajo el modelo de agricultura de autoconsumo, que se lleva a cabo en tierras de temporal y por pequeños productores, lo que representa para estos agricultores de subsistencia un reto solventar las cantidades necesarias de fertilizantes y plaguicidas químicos para una nutrición vegetal adecuada y un control de plagas y enfermedades (Kato et al*.* 2009, Massieu y Lechuga 2002).

1.3 Plagas del maíz

Entre las principales limitantes de la producción de maíz en México y en el mundo se encuentran los insectos que atacan a este cultivo y que provocan daños tanto a raíz, tallo, hojas y frutos, causando pérdidas económicas que van desde el 60 hasta el 100%. Entre los organismos que más daño causan se encuentran: el chapulín *Melanoplus differentialis* (Thomas) (Orthoptera: Acrididae), el gusano elotero *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), el pulgón del cogollo *Rhopalosiphum maidis* (Fitch) (Homptera: Aphididae), la gallina ciega *Phyllophaga vetula* (Horn) (Coleóptera: Melolonthidae), el trip *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), la diabrótica *Diabrotica balteata* (LeConte) (Coleoptera: Chrysomelidae), el gusano cogollero *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) y el gusano soldado *S. exigua* (Hubner) (Lepidoptera: Noctuidae) (Bahena y Velázquez 2012, Lesur 2005, Rodríguez y De León 2008). Para el combate contra estos insectos se aplican plaguicidas químico-sintéticos, que debido al uso excesivo de estos productos en los actuales sistemas de producción agrícola, contaminan agua, suelo y aire, provocando daños irreversibles al ambiente (Bahena y Velázquez 2012, Lesur 2005).

1.4 Hongos micorrízicos arbusculares (HMA)

La mayoría de los cultivos agrícolas, incluyendo el maíz, forman micorrizas arbusculares, que son una simbiosis mutualista entre las raíces de las plantas y ciertos hongos del Phylum Glomeromycota (Smith y Read, 2008). Los hongos micorrízicos arbusculares (HMA) son biótrofos obligados, es decir que no pueden cumplir su ciclo de vida sin la planta hospedera. La asociación HMA-planta se caracteriza por un intercambio de nutrientes. El hongo recibe azúcares generados por la planta en la fotosíntesis, mientras que la planta recibe por parte del hongo nutrientes minerales, principalmente fósforo, además de tolerancia al estrés abiótico. La asociación HMA-planta juega un papel clave en la salud y nutrición vegetal, ya sea en los ecosistemas naturales como en los agroecosistemas (Barrera 2009, Smith y Read, 2008). Por su funcionamiento en relación a la salud vegetal, los HMA son reconocidos como agentes de control biológico, principalmente en las enfermedades de las raíces causadas por hongos, oomicetes y nemátodos (Whipps, 2004). Además, la nueva perspectiva en el estudio de la multifuncionalidad de los HMA incluye procesos como la tolerancia a la salinidad, biorremediación de suelos contaminados y la inducción de resistencia sistémica en plantas (Cano 2011).

1.5 Cultivares de maíz

En cuanto al uso de cultivares o genotipos seleccionados, existen evidencias que muestran que genotipos de plantas que tienen diferencias en un solo gen, pueden tener impactos significativos en los microorganismos de la rizosfera (Berendsen et. al. 2012). Además, se sabe que cambios en el genotipo o especie de la planta o del régimen de fertilización, traen consigo modificaciones en los exudados de sus raíces, que a su vez alteran las comunidades de microorganismos de la micorrizosfera o la riqueza de las unidades taxonómicas operacionales (Aira et. al. 2010, Peiffer et. al. 2013).

1.6 Fertilización orgánica

Un buen desempeño del agroecosistema depende de las interacciones entre la diversidad de plantas y los organismos de la comunidad microbiana del suelo, respaldada por un suelo rico en materia orgánica, así, la capacidad de un cultivo de soportar y repeler el ataque de insectos plaga y enfermedades está relacionada con las propiedades físicas, químicas y especialmente biológicas del suelo (Altieri y Nicholls 2006, Kumar *et al*. 2004). Un suelo con alto porcentaje de materia orgánica y gran actividad biológica, muestra una buena fertilidad, como también cadenas tróficas complejas y microorganismos benéficos abundantes que previenen las infecciones de patógenos y los ataques de insectos herbívoros (Nicholls y Altieri 2006, Wardle *et al*. 2004). En la producción agrícola se aplican diferentes tipos de materia orgánica como mejoradores del suelo, como son estiércol de diferentes animales, abono verde, composta y vermicomposta. Existen algunos trabajos relacionados al control de distintos artrópodos plaga en plantas después de la aplicación de materia orgánica. Brown y Tworkoski (2004) reportan reducción significativa de insectos minadores en manzano utilizando composta. Alyokhin *et al*. (2005) reportan disminución significativa del escarabajo de la papa usando estiércol de res. Ponti *et al*. (2007) reportan reducción significativa de áfidos en brócoli usando composta. Morales *et al*. (2007) reportan disminución significativa de áfidos en maíz utilizando estiércol de res y composta y Arancon *et al*. (2007) reportan reducción notable de poblaciones de ácaros, chinches y áfidos en tomate y pepino usando vermicomposta (Domínguez *et al*. 2010). Los trabajos anteriores demuestran, que la fertilización orgánica, además de mejorar las condiciones físicas del suelo, funciona como controlador de algunos insectos herbívoros que se alimentan de las plantas (Nicholls y Altieri 2006).

1.7 Interacciones bióticas

Las interacciones bióticas son las relaciones que se generan entre dos o más especies, en este tipo de asociaciones los individuos involucrados pueden ser perjudicados, beneficiados o no ser afectados. Dichas interacciones se producen de la necesidad de obtener los recursos necesarios para sobrevivir (agua, nutrientes, luminosidad) y se clasifican por la afectación que tiene una especie sobre la otra, considerando un número limitado de interacciones (competencia, depredación, mutualismo, comensalismo y amensalismo). Sin embargo, estas interacciones no son tan rígidas y su relación puede alternar del antagonismo (interacción negativa de dos organismos) al mutualismo (cuando los dos organismos son beneficiados), dependiendo de los factores bióticos y/o abióticos que intervengan (del Val y Boege 2012).

1.8 Antecedentes

Hasta hace dos o tres décadas los componentes de arriba y abajo del suelo se consideraban aislados uno del otro, sin embargo, esta situación ha cambiado y en la última década las investigaciones sobre las interacciones arriba y abajo del suelo han aumentado. Actualmente, se reconoce la influencia que se ejercen mutuamente los dos componentes del suelo y el papel fundamental que juega la retroalimentación arriba-abajo en el control de los procesos y propiedades de los ecosistemas; dichas investigaciones han comprobado que las interacciones que se llevan a cabo entre las raíces, los herbívoros edáficos, los hongos mutualistas y la flora microbiana, no solo afectan el crecimiento de las plantas sino que también influyen a niveles tróficos superiores como son los herbívoros foliares, parasitoides, hiperparasitoides y polinizadores (de la Peña 2009, Rodríguez-Echeverría y de la Peña 2009, Wardle *et al*. 2004).

Las interacciones entre los HMA y los artrópodos plaga han sido menos estudiadas y parecen ser más complejas que las encontradas con los HMA y las enfermedades, aunque se sabe que ambos grupos de organismos (HMA y artrópodos) se pueden afectar mutuamente de forma indirecta y a veces de forma directa (Ghering y Bennett 2009). Los HMA tienen efectos significativos en todos los aspectos del desarrollo de los insectos herbívoros, tales como consumo de alimento, tasa de crecimiento, fecundidad, sobrevivencia y densidad de insectos herbívoros. Estudios con insectos masticadores especialistas e insectos chupadores como los pulgones han mostrado un aumento en el desempeño de la plaga en plantas micorrizadas, mientras el desempeño de insectos masticadores generalistas se ve reducido en plantas con micorriza, sin embargo, la magnitud y dirección de estos efectos dependen del modo de alimentación, del tipo de dieta del insecto y de la especie del hongo (Koricheva *et al*. 2009). En el caso específico de esta investigación, de acuerdo a algunos autores la fertilización orgánica favorece el desarrollo de los HMA (Albertsen et al. 2006, Gryndler et al. 2006, Yu et al. 2013), pero aún falta información sobre cómo afecta la fertilización orgánica las interacciones entre los HMA y las plagas de artrópodos.

1.9 Justificación

Debido a la problemática nacional y mundial por la contaminación de agua, suelo y aire, causada por el uso excesivo de agroquímicos utilizados en los actuales sistemas de producción agrícola, es prioritario buscar modelos alternativos sostenibles. Se propone en este trabajo de investigación, explorar el uso de la fertilización orgánica y los hongos micorrízicos arbusculares (HMA) como parte fundamental de un Manejo Agroecológico de Plagas y Enfermedades (MAPE) en el cultivo del maíz. La relevancia de este proyecto radica en que es una investigación original, ya que a la fecha no existen trabajos que aborden en un mismo estudio las interacciones entre HMA, insectos herbívoros y cultivares de maíz, bajo diferentes escenarios de fertilización orgánica.

CAPÍTULO 2. HIPÓTESIS Y OBJETIVOS (GENERAL Y PARTICULARES)

HIPÓTESIS.

Una mayor cantidad de materia orgánica en el suelo, proporciona una mayor cantidad de microorganismos benéficos, que proveen de más nutrientes a la planta y esto a su vez le confiere una mayor tolerancia contra la herbivoría por insectos.

OBJETIVOS (GENERAL Y PARTICULARES).

Objetivo general:

Evaluar los efectos de la fertilización orgánica en las interacciones bióticas entre hongos micorrízicos arbusculares (HMA), insectos herbívoros y cultivares de maíz.

Objetivos particulares:

1) Evaluar el efecto de diferentes tipos de fertilización orgánica en el desempeño vegetal del maíz y sus microorganismos asociados.

2) Evaluar el efecto de la fertilización orgánica y mineral en las interacciones bióticas maíz-HMA-insectos herbívoros.

3) Evaluar el efecto de diferentes tipos de HMA en las interacciones bióticas maíz-HMA-insectos herbívoros.

CAPÍTULO 3. Manuscrito 1.

Organic fertilizers alter the composition of pathogens and arbuscular mycorrhizal fungi in maize roots.

Aguilar R., Carreón-Abud Y, López-Carmona D, Larsen J.

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Organic fertilizers alter the composition of pathogens and arbuscular mycorrhizal fungi in maize roots

Ricardo Aguilar[1,2] | Yazmin Carreón-Abud[2] | Dante López-Carmona[1] | John Larsen[1]

[1]Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México (UNAM), Morelia, Michoacán, México

[2]Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Gral. Francisco J. Múgica S/N, Ciudad Universitaria, Morelia, Michoacán, México

Correspondence
John Larsen, Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México (UNAM), Morelia, Michoacán, México.
Email: jlarsen@cieco.unam.mx

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Abstract

Roots of agricultural crops, including maize, are hosts of different microorganisms, many beneficial, like plant growth and health-promoting arbuscular mycorrhizal fungi (AMF), as well as pathogens including *Pythium*, *Polymyxa* and *Microdochium*. To improve crop nutrition and health, profound knowledge is required regarding how agricultural practices affect field populations of root-associated microorganisms. Hence, the objective of this work was to evaluate the effect of crop genotype and organic fertilizers on the plant growth performance of maize and their root-associated microorganisms. The experiment was conducted as a fully factorial greenhouse pot experiment with maize cultivars (two land races and two hybrids) and organic fertilizers (green manure, cow manure and compost) as the two main factors. Plants were harvested 8 weeks after sowing. In general, the different maize cultivars responded similarly to the applications of the organic fertilizers. Cow manure and compost increased plant growth, whereas green manure had limited effect on plant growth. Root colonization with AMF was reduced by green manure with rape. Infection with the root pathogens *Pythium* and *Polymyxa* was reduced by all organic fertilizers, whereas in contrast, infection with *Microdochium* increased with the majority of the organic fertilizers applied. In conclusion, both maize genotype and organic fertilizers affect the abundance of AMF and root pathogens in maize, which should be considered when developing management strategies of these root-inhabiting microorganisms.

KEYWORDS
arbuscular mycorrhizal fungi, maize genotype, organic fertilization, root pathogens

1 | INTRODUCTION

Plant roots are hosts of an abundant and diverse population of fungi and fungal-like organisms, including plant growth and health-promoting arbuscular mycorrhizal fungi (AMF) (Smith & Read, 2008) and pathogens such as oomycetes, protozoans and fungi (Munkvold & White, 2016). AMF form symbiosis with most crops in both natural and agricultural ecosystems (Smith & Read, 2008) providing important ecosystem services related to host nutrition and health (Gianinazzi et al., 2010). On the contrary, root pathogens can cause severe damage to most crops in terms of root-rot and growth depressions. However, root diseases may be difficult to diagnose due to the hidden belowground status, why they frequently are confused with nutrient or water deficiency.

Mexico is the origin of maize domestication with a diverse genetic population and many land races as well as hybrids being cultivated both by small holders and at industrial scale (Sanchez, Goodman, & Stuber, 2000). Maize naturally forms mycorrhizal associations with AMF and is considered mycotrophic (Plenchette, Fortin, & Furlan, 1983). Common maize root pathogens include nematodes, different oomycete *Pythium* spp, the fungus like protozoa *Polymyxa graminis* and several true fungi such as *Fusarium verticilloides* (Munkvold & White, 2016).

Management of plant growth and health-promoting root fungi and pathogens in agroecosystems requires a profound knowledge about their ecology and how they respond to agricultural practices such as crop rotation, tillage, pest control methods and fertilization (Larsen, Rincón, Gonzalez-Esquivel, & Gavito, 2014).

Organic fertilization, which includes the use of animal manure, green manure and various types of compost, is the cornerstone of organic agriculture and is also employed as complementary fertilization in conventional agriculture combined with mineral fertilizers.

In general, root pathogens are reduced by application of organic fertilizers (Hoitink & Boehm, 1999; Noble & Coventry, 2005), but may also increase as reported by Yu, Nicolaisen, Larsen, and Ravnskov (2013), where the abundance of *Olpidium brassicae* (Fungi *insertae sedis*) in pea roots increased when an organic fertilizer was applied to the soil.

Organic and mineral fertilization have been shown to, respectively, increase and decrease the formation of mycorrhizal associations in agroecosystems (Gryndler et al., 2006), and in general, AMF seems to thrive in organic matter (Albertsen, Ravnskov, Green, Jensen, & Larsen, 2006; Gosling, Hodge, Goodlass, & Bending, 2006; Ravnskov, Larsen, Olsson, & Jakobsen, 1999; Yu et al., 2013).

Effects of green manure on root fungi have been shown to depend on type of green manure and root fungi in question (Detheridge et al., 2016). However, there is a general consensus that rape-based green manure can reduce both AMF and root pathogens, most likely due to the liberation of the isothiocyanates during the decomposition process (Larkin, 2013). Crop genotype may also determine the abundance and composition of root fungi (Aira, Gómez-Brandón, Lazcano, Bååth, & Dominguez, 2010; Peiffer et al., 2013). In general, maize associate with AMF and may promote host plant growth (Gavito & Varela, 1995), but landraces have been environmentally selected with the adaptation of native populations of AMF (Sangabriel-Conde, Negrete-Yankelevich, Maldonado-Mendoza, & Trejo-Aguilar, 2014), which may provide more compatible mycorrhizal associations. Information on the response of maize genotypes to root pathogens is limited.

The main objective of this study was to examine the response of AMF and root pathogens to organic fertilizers including compost, animal and green manure in different maize genotypes including hybrids and land races commonly grown in Mexican maize agroecosystems.

2 | MATERIALS AND METHODS

2.1 | Experimental design

The experiment had a completely randomized factorial block design. Following factors were examined: (i) maize genotypes (four levels: two hybrids (DK-2061 and DK 2042 from DeKalb) and two landraces (Elotes Occidentales and Criollo Odilon)) and (ii) fertilization (eight levels: without, mineral, compost, vermicompost, cow manure, green manure in terms of vetch, oat or rape). Each treatment had four replicates, giving a total of 128 experimental units.

2.2 | Experimental set-up

2.2.1 | Soil

Soil was obtained from the experimental field station of the National Agricultural University of Mexico, Campus Morelia, Michoacán, Mexico. The soil texture was clayish consisting of 53.2% clay, 27.3% silt and 19.5% sand. The chemical characteristic of the soil was 2.7% organic matter, 23.2 mg/kg inorganic nitrogen, 5.8 mg/kg available phosphorus (Olsen P) and pH (H_2O) 7.3. The soil was mixed with quartz sand (1:1, w/w) and sterilized twice by autoclaving (15 lbs, 120°C) during an hour. In each pot, 800 g soil-sand mix was placed in a plastic bag to avoid leaching from the pots. Hereafter, three seeds were sown, but were thinned to one uniform seedling in each pot after seedling emergence.

2.2.2 | Application of mineral and organic fertilizers

In the mineral fertilization treatment, a full basic mineral fertilization was applied to each pot before sowing maize seeds corresponding to the following amounts of mineral salts (mg/kg soil): K_2SO_4 (75), $CaCl_2 \times 2H_2O$ (75), $CuSO_4 \times 5H_2O$ (2.1), $ZnSO_4 \times 7H_2O$ (5.4), $MnSO_4 \times H_2O$ (10.5), $CoSO_4 \times 7H_2O$ (0.39), $MgSO_4 \times 7H_2O$ (45), $Na_2MoO_4 \times 2H_2O$ (0.18), KH_2PO_4 (439.3) and NH_4NO_3 (600). Regarding the cow manure, compost and vermicompost 100 ml was applied to each pot of the respective treatments 1 month before sowing of maize seeds. Each of the green manure crops (50 seeds per pot) was grown for 8 weeks in their designated pot after which the root and shoot biomass were cut in 1-cm pieces and incorporated into the soil. Hereafter, the pots were left for decomposition of the plant biomass for another 8 weeks under greenhouse conditions and watered as needed to maintain the soil moist. Information on the amount of biomass added and content of N and P in the different types of organic fertilizers as well as their origin is presented in Table 1.

2.3 | Plant growth conditions, harvest and analyses

Plants were grown under greenhouse conditions with minimum and maximum temperature of 15°C and 35°C, respectively. Plants were watered to 70% of the water-holding capacity, which was maintained throughout the experiment by watering by weight on a daily basis. Nitrogen (30 mg) in terms of NH_4NO_3 was applied every week 3 weeks after sowing and until the last week before harvest. Eight weeks after sowing plants were harvested. Roots were gently washed free of growth substrate and the shoot separated from the root. Dry weights of shoots and roots were obtained after drying at 70°C for 48 hr. Prior drying of roots, they were cut into 5- to 10-mm segments and a representative two-gram root subsample was taken. Root subsamples were cleared and stained according to Phillips and Hayman (1970) and examined for AMF root colonization and pathogen root infection with *Pythium* (oospores), *Polymyxa* (sporangia) and *Microdochium* (dark spore clusters) using the line-intercept method as described by Giovannetti and Mosse (1980).

2.4 | Statistics

Two-way analyses of variance with maize genotypes and fertilization as factors for all variables were intended. Bartlett test was used to verify variance homogeneity. For the variables shoot and root dry weight, variance homogeneity was not obtained, why the nonparametric Kruskal–Wallis test with subsequent post hoc ANOVA treatment comparisons with the Dunn test was employed for these variables. For all other variables, variance homogeneity was obtained after arcsine transformation and here treatment means were compared with the post hoc ANOVA LSD test. Statistical analysis was performed with the software Statgraphics Centurion XVII for the two-way ANOVA analyses, and the JMP Software from SAS was used to perform the Kruskal–Wallis test.

3 | RESULTS

3.1 | Shoot dry weight

A significant effect of the nonparametric Kruskal–Wallis test was obtained for shoot dry weight (p = .0001; Table 2). In general, all four maize genotypes responded similarly to the different types of organic fertilization (Table 2). Mineral fertilization caused the highest shoot dry weight in all maize genotypes. All three types of green manure had no effect on maize shoot dry weight except in the hybrid maize DK-2061 where green manure with vetch slightly increased maize shoot dry weight (Table 2). Cow manure, compost and vermicompost also increased shoot dry weight of all maize varieties, although not as much as that of mineral fertilization. Among the compost, vermicompost increased maize shoot dry weight higher than that of the cow manure and compost except Criollo Odilon where no difference was found between cow manure, compost and vermicompost (Table 2).

3.2 | Root dry weight

A significant effect of the nonparametric Kruskal–Wallis test was also obtained root dry weight (p = .0001; Table 3). Also for root, dry weight mineral fertilization caused the highest dry weight in all maize genotypes. All three types of green manure had no effect on root dry weight except in the maize land race Criollo Odilon, where green manure with vetch also increased root dry weight almost to the same extent as that of mineral fertilization (Table 3). Cow manure, compost and vermicompost also increased root dry weight of all maize varieties, although not as much as that of mineral fertilization (Table 3).

TABLE 1 Content of N and P in the organic fertilizers and the total N and P added to the respective treatments

Fertilizer	Biomass (g dwt.)	N conc.(mg/g)	Total N(mg)	P conc.(mg/g)	Total P(mg)	Reference
Green manure (oat)	19.9	11.4	227	5.3	105	Ortiz (2015)
Green manure (rape)	7.9	11.0	87	5.5	43	Ortiz (2015)
Green manure (vetch)	12.9	20.0	258	6.4	83	Ortiz (2015)
Cow manure[a]	46	18.4	846	17.3	796	Gonzálvez and Pomares (2008)
Compost[b]	65	17.0	1,105	18.0	1,170	Arancon, Babenko, Cannon, Galvis, and Metzger (2008)
Vermicompost[c]	56	13.0	728	27.0	1,512	Arancon et al. (2008)
Mineral	–	–	210	–	150	See Material and Methods

[a]Fresh dried cow manure (collected from a local farmer).

[b]Based on biosolids and waste of water hyacinth (*Eichhornia crassipes*) green waste.

[c]Based on cow manure with red wiggler worms (*Eisenia foetida*). Values of N and P concentrations in the organic fertilizers are based on the literature estimates. Compost and vermicompost were purchased from local agro-companies. dwt, dry weight.

TABLE 2 Shoot dry weight (g) of four maize varieties grown for 8 weeks with different types of organic fertilization and mineral fertilization or without any fertilization (n = 4). Different letters indicate significant differences between treatments

	Maize varieties			
Fertilizer	Elotes Occ.	Criollo Od.	DK-2061	DK-2042
Without	2.35^{j-l}	2.42^{jkl}	1.50^{mn}	1.83^{lmn}
Mineral	7.24^{a}	6.63^{ab}	6.53^{b}	5.31^{c}
Vetch*	2.70^{h-k}	2.71^{h-k}	2.43^{j-l}	2.22^{k-l}
Oat*	1.99^{lm}	1.85^{lmn}	1.21^{n}	1.19^{n}
Rape*	2.30^{k-l}	1.85^{lmn}	1.49^{mn}	1.29^{n}
Cow manure	3.63^{efg}	3.47^{fg}	3.04^{g-j}	3.35^{f-i}
Compost	3.74^{def}	3.39^{fgh}	3.57^{efg}	3.63^{efg}
Vermicompost	4.35^{d}	3.92^{def}	4.25^{de}	4.25^{de}

*As a green manure precrop; Occ, Occidentales; Od., Odilon. Kruskal–Wallis test $p < .001$.

TABLE 3 Average root dry weight (g) of four maize varieties grown for 8 weeks with different types of organic or mineral fertilization or without any fertilization ($n = 4$). Different letters indicate significant differences between treatments

	Maize varieties			
Fertilizer	Elotes Occ.	Criollo Od.	DK-2061	DK-2042
Without	1.24^{ghi}	1.45^{f-i}	0.90^{i}	1.21^{ghi}
NPK	2.88^{abc}	3.54^{a}	3.13^{ab}	2.99^{abc}
Vetch*	1.82^{e-h}	2.73^{bcd}	1.55^{f-i}	1.50^{f-i}
Oat*	1.28^{ghi}	1.28^{ghi}	1.14^{hi}	0.97^{i}
Rape*	1.43^{f-i}	1.45^{f-i}	1.24^{ghi}	0.91^{i}
Cow manure	1.85^{efg}	2.08^{def}	1.90^{efg}	2.03^{def}
Compost	2.04^{def}	2.33^{cde}	1.90^{efg}	2.03^{def}
Vermicompost	2.05^{def}	2.65^{bcd}	2.11^{def}	2.50^{b-e}

*As a green manure precrop; Occ, Occidentales; Od., Odilon. Kruskal–Wallis test $p < .001$.

3.3 | AMF root colonization

Significant effects of both individual factors maize genotype ($p = .0000$) and fertilization ($p = .0002$) were obtained for AMF root colonization, but no interaction between main factors was obtained ($p = .4380$; Figure 1). AMF root colonization was higher in the two hybrids than in the landraces (Figure 1a). None of the fertilizers examined affected AMF root colonization except rape as green manure which caused a moderate reduction in AMF root colonization (Figure 1b).

3.4 | Root pathogen infection

A significant interaction ($p = .0064$) between maize genotype and fertilization was obtained for *Pythium* root infection in terms of oospore abundance in roots (Table 3). Also significant effect was obtained for both single factors: maize genotype ($p = .0061$) and fertilization ($p = .0000$). Maize genotypes harboured different levels of *Pythium* infection, but this effect depended on type of fertilization. In plants fertilized with mineral fertilization, *Pythium* root infection was lower in the two hybrids compared with the landraces. In general, all three green manure fertilizers (vetch, oat and rape) strongly reduced *Pythium* root infection independent of maize genotype, when compared with the treatment without fertilization (Figure 2). Also cow manure, compost and vermicompost reduced *Pythium* root infection to some extent but with no clear pattern (Figure 2).

Significant effects of the factor fertilization were obtained in terms of *Polymyxa* ($p = .0006$; Figure 3) and *Microdochium* ($p = .0033$; Figure 4) root infection (Table 3). All types of fertilizers including mineral reduced *Polymyxa* root infection independent of maize genotype (Figure 3). In contrast, all organic fertilizers increased *Microdochium* root infection independent of maize genotype though only significantly with the green manure vetch and oat. Mineral fertilization had no effect on *Microdochium* root infection (Figure 4).

4 | DISCUSSION

Main results from our study showing that the native field populations of AMF and the root pathogens *Pythium*, *Polymyxa* and *Microdochium* responded differently to the factors examined (maize genotype and fertilization) provide useful information on possible management of AMF and root pathogens in maize agroecosystems.

Arbuscular mycorrhizal fungi are known to play an important role in agroecosystems in relation to crop health and nutrition (Gianinazzi et al., 2010), and they have been shown to respond to agricultural practice such as crop genotype, tillage, pesticides and fertilization (Larsen et al., 2014). Here, we show that AMF root colonization was higher in hybrid maize than in landrace maize, which is in contrast to Gavito and Varela (1993), but in both cases, only a few genotypes were included. Hence, future studies comparing mycorrhiza formation in landrace and hybrid maize should include more genotypes in order to improve the knowledge about mycorrhiza compatibility in hybrid maize compared with landraces. On the other hand, in wheat, loss of mycorrhiza compatibility has been reported for hybrid genotypes (Hetrick, Wilson, & Cox, 1992).

Mineral fertilization especially with P has been shown to reduce formation of the AMF symbiosis (Thomson, Robson, & Abbott, 1992). However, in the present study, mineral fertilization had no effect on AMF root colonization. Perhaps, this difference is due to the fact that the soil employed in the present study was very limited in P for maize plant development. Critical values of soil P for optimal maize development have been reported to be 15 mg P/g soil (Tang et al., 2009), which is substantial higher than the soil P level of 5.8 mg P/g soil used in the present study. In this case, applying P will allow the host to better develop and as a consequence allocate more C to the mycorrhizal association.

In general, AMF seems to thrive in organic matter (Albertsen et al., 2006; Gosling et al., 2006; Ravnskov et al., 1999; Yu et al., 2013); however, the organic fertilizers examined in the present study had no effect on AMF root colonization, except for green manure in terms of rape, which caused a decrease in the AMF root colonization. The reason for this discrepancy may be due to differences in type of organic matter examined related to elevated levels of soil P levels, which is known to limit formation of mycorrhizal associations as mentioned above. On the other hand, the above-mentioned effect of rape suppression of AMF root colonization is most likely due to the liberation of allelopathic compounds such as isothiocyanates during the decomposition process (Larkin, 2013).

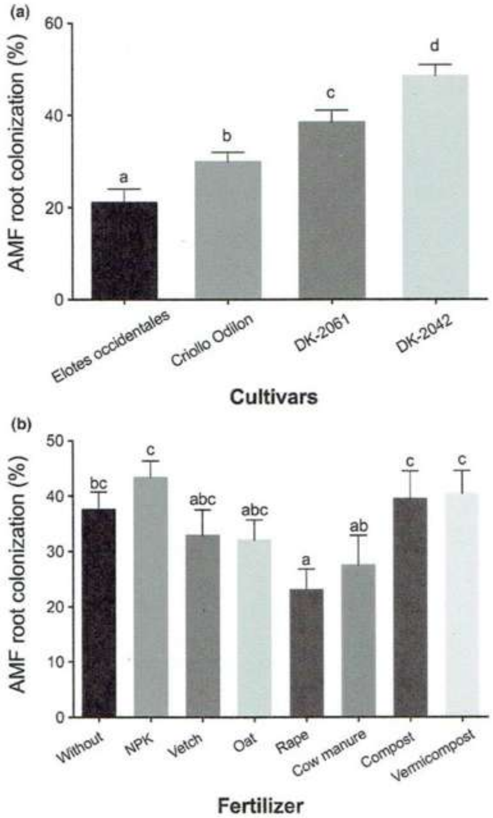

FIGURE 1 Factor treatments means of AMF root colonization (%) of 8-week-old maize in terms of the factors maize genotype (Elotes occidentales, Criollo Odilon, DK-2061 and DK-2042) (a) and fertilization (without, mineral (NPK), vetch, oat and rape in terms of green manure, cow manure, compost and vermicompost (Table 1) (b). Different letters indicate significant differences between treatments. Error bars represent standard error of the mean

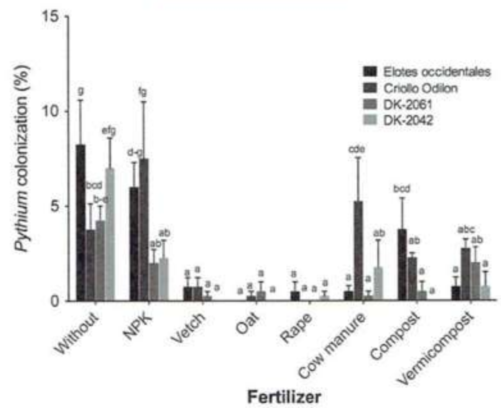

FIGURE 2 *Pythium* root infection (%) in terms of oospores in maize grown for 8 weeks as affected by maize genotype (Elotes occidentales, Criollo Odilon, DK-2061 and DK-2042) and fertilization (without, mineral (NPK), vetch, oat and rape in terms of green manure, cow manure, compost and vermicompost (Table 1). Different letters indicate significant differences between treatments. Error bars represent standard error of the mean

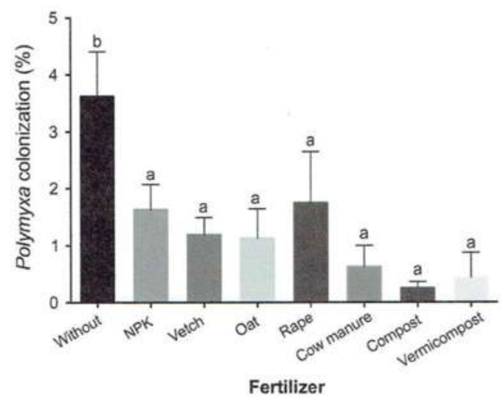

FIGURE 3 Factor treatment means of *Polymyxa* root infection in maize roots in terms of in terms of sporangia for the factor fertilization (without, mineral (NPK), vetch, oat and rape in terms of green manure, cow manure, compost and vermicompost (Table 1). Different letters indicate significant differences between treatments. Error bars represent standard error of the mean

The differential effects of root pathogens from organic fertilizers may be linked to their respective biology. The pathogens *Pythium* and *Polymyxa*, which are, respectively, hemi-biotrophs and obligate biotrophs, were reduced by organic fertilizers, whereas *Microdochium* root colonization, which is a facultative saprotroph, was increased by all organic fertilizers. Likewise, Yu, Nicolaisen, Larsen, and Ravnskov (2012) showed that saprotrophic fungi were more abundant in the senescence plant growth phase in pea roots, where more dead organic material is available.

Root pathogens have been found to be suppressed by compost or amendment with organic matter in general (Bonaomi, Antignani, Capodilupo, & Scala, 2010; Hoitink & Boehm, 1999; Litterick, Harrier, Wallace, Watson, & Wood, 2004; Noble & Coventry, 2005), which was supported by the results from the present study showing that *Pythium* and *Polymyxa* were suppressed by all types of organic matter. The mode of action of the organic matter suppression may be related to biocontrol from other microorganisms such as competition for nutrients and antibiosis (Hoitink & Boehm, 1999), as well as induction of plant resistance (Lievens, Vaes, Coosemans, & Ryckeboer, 2013) and generation of toxic N containing volatiles such as ammonia and nitrous oxide (Lazarovits, 2001). Indicators of disease-suppressive effects of organic amendments seem

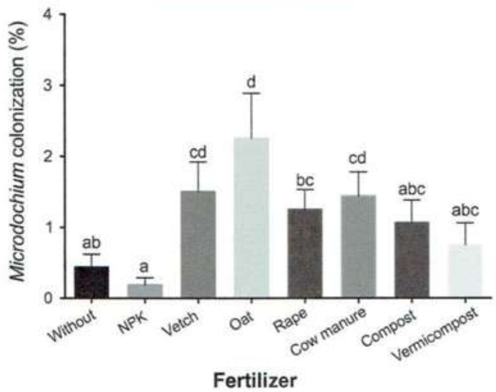

FIGURE 4 Factor treatment means of *Microdochium* root infection in terms dark spore clusters in maize in 8-week-old maize in terms of the factor fertilization (without, mineral (NPK), vetch, oat and rape in terms of green manure, cow manure, compost and vermicompost (Table 1). Different letters indicate significant differences between treatments. Error bars represent standard error of the mean

to be linked with total microbial biomass and activity (Hadar & Papadopoulou, 2012), but also chemical characteristics such as humic fractions from compost have been shown to be of importance in disease suppression of compost (Pascual, Garcia, Hernandez, Lerma, & Lynch, 2002).

Green manure has been shown to reduce several root pathogens including nematodes, oomycetes and true fungi in terms of incorporation of a previous crop such as oat, vetch and different Brassicaceae crops (Larkin, 2013). This is clearly supported by the present study where all three green manure crops suppressed both *Pythium* and *Polymyxa* root infection levels. Variables measured in the present study do not provide information on possible mode of action of the observed green manure pathogen suppression. However, possible mode of action of pathogen suppression may be linked with production of allelopathic compounds (Larkin, 2013).

In conclusion, main results from the present study reveal that both maize genotype and organic fertilizers affect the abundance of AMF and root pathogens in maize. In general, organic fertilizers differentially affect root fungi from different functional groups, which seems to be linked to the biology of the pathogen in question.

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CAPÍTULO 4. Manuscrito 2.

The influence of mineral and organic fertilization on multi-trophic maize-mycorrhiza-*Spodoptera frugiperda* interactions

Ricardo Aguilar[1,2], Ek del Val[1], Omar Real-Santillan[1], Tsiri Diaz[1], Yazmin Carreon-Abud[2] and John Larsen[1]

[1]Instituto de Investigaciones en Ecosistemas y Sustentabilidad, México, Antigua Carretera a Pátzcuaro 8701, Col. Ex Hacienda San José de la Huerta, 58190 Morelia, Michoacán, México.

[2]Facultad de Biología, Universidad Michoacana de San Nicolas de Hidalgo, Gral. Francisco J. Múgica S/N, Ciudad Universitaria, 58030 Morelia, Michoacán, México.

Abstract

The foliar herbivory produced by the fall armyworm *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) causes great yield loss in maize agroecosystems. Conventionally, the control of this insect pest is based on chemical synthetic insecticides, but given the environmental impact that this practice causes, it is necessary to find a more sustainable pest management alternative. In two greenhouse pot experiments, we evaluated how mineral and organic fertilization influence multi-trophic maize-mycorrhiza-*S. frugiperda* interactions. In the first experiment, the effect of mineral and organic fertilization on the herbivory caused by *S. frugiperda* in native and hybrid maize grown in unsterilized soil was evaluated. In the second experiment, the effect of soil disinfection on the herbivory caused by *S. frugiperda* in native and hybrid maize with mineral and organic fertilization was evaluated. In general, our results show that the herbivory did not affect the maize mycorrhizal association, and similarly, eliminating the native arbuscular mycorrhizal fungi (AMF) by soil sterilization did not affect the performance of *S. frugiperda* either. Mineral and organic fertilization increased maize plant biomass, which also resulted in greater larvae biomass, whereas no effect on the herbivory was observed. Independently of the fertilization and the soil disinfection, the larvae grew more eating leaves from the native maize genotype than from the hybrid. Conversely, soil disinfection caused an increase in the shoot dry weight of the hybrid, while with the native maize genotype the soil disinfection shoot dry weight was not affected. In conclusion, our results show that the damage caused by the herbivory from *S. frugiperda* was not affected by fertilization or the soil sterilization.

Key words: Fertilization, arbuscular mycorrhizal fungi, maize genotypes, Fall Armyworm.

Introduction

Maize is among the most cultivated cereal crops in the world, and it is a crop of great importance in Mexico, since around 60% of the national production is for human consumption (Massieu and Lechuga 2002). In 2012, Mexico occupied the fourth place worldwide (3% of the total), with a production of 22.1 million tons (Anonymous, 2012). In Mexico maize land races are diverse (between 41 and 65 have been described) and more than half of the planted surface is being cultivated with these genotypes with the traditional agriculture model for self-consumption by smallholders (Kato *et. al.* 2009). In these agroecosystems application of agrochemicals like mineral fertilizers and pesticides is the main method for crop nutrition and pest control, respectively (Kato *et. al.* 2009, Massieu and Lechuga 2002).

Among the main limitations of maize production are arthropods that attack this crop and cause damage to the roots, stalks, leaves and fruits, causing economic losses that range from 60% to 100%. The organisms that cause the most damage include the grub worm *Phyllophaga vetula*

(Horn) (Coleóptera: Melolonthidae), the Western corn rootworm *Diabrotica balteata* (LeConte) (Coleoptera: Chrysomelidae), thrip *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), corn leaf aphid *Rhopalosiphum maidis* (Fitch) (Homptera: Aphididae), the fall armyworm *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), the beet armyworm *S. exigua* (Hubner) (Lepidoptera: Noctuidae), the corn earworm *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) and grasshopper *Melanoplus differentialis* (Thomas) (Orthoptera: Acrididae) (Bahena y Velázquez 2012, Lesur 2005, Rodríguez y De León 2008). To combat these arthropods, synthetic chemical pesticides are applied, but owing to the excessive use of these products in the current systems of agricultural production, they pollute the water, soil and air, causing irreversible environmental damage (Bahena and Velázquez 2012, Lesur 2005).

In light of this situation, it is a priority to find alternative sustainable models, so this research study proposes to explore the use of organic fertilization as a fundamental part of the agroecological management of pests and diseases (AMPD) in the maize crop. This type of management indicates that functionality of an agroecosystem can be increased by means of managing two main components: 1) Improving the regulation of pests through crop diversification, cultural practices, biological control and habitat modification and 2) Improving soil fertility through the use of biofertilizers, cover crops, green manure, crop rotation and organic fertilization (Altieri and Nicholls 2003).

Organic fertilization, which includes the use of animal manure, green manure and different types of compost, is the basis of organic agriculture, where modifications of organic matter have shown, on one hand, the suppression of root pathogens and, on the other, the promotion of arbuscular mycorrhizal fungi (Aguilar et. al. 2017, Albertsen et. al. 2006, Gosling et. al. 2006, Noble and Coventry 2005, Raaijmakers et. al. 2009, Yu et. al. 2013).

The performance of the agroecosystem depends on the interactions between plant diversity and soil microbial communities, supported by a soil rich in organic matter that promotes the crops' capacity to withstand and repel attacks from insect infestations and diseases that are related to the physical, chemical and biological properties of the soil (Altieri and Nicholls 2006, Kumar *et al*. 2004). Soils with high level of organic matter promote biological activity, as well as complex trophic chains and the abundance of beneficial microorganisms that prevent infections from pathogens and insect attacks (Nicholls and Altieri 2006, Wardle *et al*. 2004). In addition, the application of organic matter like manure and compost has resulted in the reduction of damage caused by various arthropod infestations (Domínguez *et al*. 2010). In this way, organic fertilization, besides improving the physical conditions of the soil, acts as a mean to control some herbivorous insects (Nicholls and Altieri 2006).

For agroecological management, it is important to consider how agricultural practices like tillage, biological control, crop rotation and organic fertilization affect multi-trophic plant-microorganism-insect interactions (Larsen *et al.* 2015). Hence, the objective of the present work was to investigate how mineral and organic fertilization influence the multi-trophic interactions between maize, mycorrhiza and the fall armyworm with the main hypothesis that compost reduces and promotes the development of *S. frugiperda* and the mycorrhizal relationship respectively.

Materials and methods

Experiment one

Experimental design

This experiment had a completely random factorial design. The analyzed factors included the following: 1) Maize cultivars (two levels: a hybrid, DK-2061, and a native, Criollo Odilón); 2) Fertilization (three levels: without fertilizer, organic (vermicompost) and mineral (NPK); and 3) Insect, *S. frugiperda* (two levels: with and without insects). Each treatment had six repetitions, for a total of 72 experimental units.

Experimental setup

The soil was obtained from the experimental field of the Autonomous University of Chapingo, the Morelia campus, Michoacán, Mexico. The soil texture was clayey, consisting of 53.2% clay, 27.3% silt and 19.5% sand. The chemical characteristics of the soil were 2.7% organic matter, 23.2 mg kg^{-1} of inorganic nitrogen, 5.8 mg kg^{-1} of available phosphorus (Olsen P), with a pH of 7.3 (H_2O). Each pot contained 1.2 kg of the soil-sand mixture (1:1, v/v) in which three seeds were placed. When the seeds emerged, two were removed in order to leave only seedlings of similar size. For the NPK treatment, a basic mineral fertilization was applied to each pot, corresponding to the following quantities of mineral salts (mg kg^{-1} soil): K_2SO_4 (75), $CaCl_2$ x $2H_2O$ (75), $CuSO_4$ x $5H_2O$ (2.1), $ZnSO_4$ x $7H_2O$ (5.4), $MnSO_4$ x H_2O (10.5), $CoSO_4$ x $7H_2O$ (0.39), $MgSO_4$ x $7H_2O$ (45), Na_2MoO_4 x $2H_2O$ (0.18), KH_2PO_4 (439.3) y NH_4NO_3 (600). In relation to the vermicompost, 100 ml was applied to each pot.

Conditions of plant growth, harvest, and analysis

The plants were grown under greenhouse conditions, with a minimum and maximum temperature of 10 ^oC and 30 ^oC, respectively. Watering was done by weighing to 70% of the soil field capacity, which was maintained throughout the experiment with daily watering. Four weeks after planting, the plants were moved to a growth chamber with a controlled temperature of 24 ^{o}C. Here the damage caused by the fall armyworm (*S. frugiperda*) in terms of foliar herbivory was evaluated measuring the initial and final weight of the insect larvae. Each plant was completely covered with a cloth to confine the larvae area. Five fall armyworm larvae between instar stages L2 and L3 were placed in each plant.

Harvesting was performed 10 days after the placement of the insects. The harvest consisted of washing the roots to remove excess substrate, separating the aerial part from the root and weighing each part's fresh weight. Afterwards, the aerial and radical parts were oven dried at 70 ^{o}C for 48 hours. Before drying the roots, they were cut into 5- to 10-mm segments, and representative 2-g subsamples of the root were taken. The subsamples were cleared and stained according to the Phillips and Hayman method (1970), for later evaluating the percentage of radical colonization by arbuscular mycorrhizal fungi using the grid-line intercept method described by Giovanetti and Mosse (1980). The variables to be measured were as follows: aerial dry weight, radical dry weight, percentage of radical colonization by arbuscular mycorrhizal fungi and the performance of the larvae.

For obtaining the insects, fall armyworm offspring were used (*S. frugiperda*), which are maintained in the Biotic Interactions in Altered Habitats Laboratory in the Institute of Ecosystems and Sustainability Research (IIES in the Spanish abbreviation).

Statistical analysis

The statistical analysis were performed by means of the GLM (generalized linear model) in program R with the factors Fertilizer, Cultivar and Insect, as well as their interactions with all the measured variables (*n*=6).

Experiment two

Experimental design

This experiment had a completely random factorial design. The examined factors included the following: 1) Cultivars of maize (two levels: hybrid, DK-2061 and native, Criollo Odilón; 2) Fertilization (two levels: organic (vermicompost) and mineral (NPK); 3) insect, *S. frugiperda* (two levels: without and with insects); and 4) soil (two levels: disinfected and not disinfected). Each treatment had six repetitions, for a total of 96 experimental units.

Experimental setup

All the biological materials (maize and insects), soil and fertilizers used in experiment two were the same as in experiment one described in the previous section.

Half the substrate was sterilized twice in an autoclave for an hour at 15 lbs. of pressure and 120 ^{o}C.

As in experiment one, each pot contained 1.2 kg of the soil-sand mixture, in which three seeds were placed. After a week, two were removed in order to leave only seedlings of similar size.

Conditions of plant growth, harvest and analysis

The plants were placed under greenhouse conditions, with a minimum and maximum temperature of 15 ^{o}C and 35 ^{o}C, respectively. The plants were watered at 70% of their field capacity, which was maintained throughout the experiment with daily watering by weight. The maize seeds were sown in 1.5 liter pots with 1.2 kg of substrate made of experimental field soil as in experiment one mixed with sand in a 1:1 ratio.

In this experiment, the damage caused by the fall armyworm (*S. frugiperda*) was also evaluated in terms of foliar herbivory measured in terms of biomass reduction. Six weeks after sowing, three fall armyworm larvae between instar stages L2 and L3 were placed in each plant, to be collected 14 days after the placement of the insects. Before placing the larvae, each plant was covered with a cloth to assure that the larvae could not escape.

Two weeks after the placement of the larvae, the harvest was performed with the same protocol as in experiment one. The variables measured were the shoot and root dry weight, the percentage of root colonization by the AMF and larval weight as performed in experiment one.

Statistical analysis

The statistical analysis was performed through the GLM (generalized liner model) in program R with the factors Fertilizer, Cultivar, Insect and Sterilization and their interactions with all the measured variables (aerial dry weight, root dry weight, percentage of colonization by AMF and larvae weight (n=6).

3. Results

Experiment one

Significant results were obtained for the Fertilizer factor in all the measured variables (Table 1). For the Cultivar factor, significant effects were obtained for the shoot and root dry weight. For the shoot dry weight, the Fertilizer x Insect interaction was also significant (Table 1).

Mineral fertilization resulted in the highest shoot dry weight, mineral fertilization intermediate and plants without fertilization lowest (Figure 1a). The damage by *S. frugiperda* herbivory was more noticeable in the plants without fertilizers (Figure 1a). The general plant performance, regarding the shoot dry weight (Figure 1b) and the root dry weight (Figure 1d) of the two tested cultivars, in general was higher in the criollo than in the hybrid, independently of the other evaluated factors. For the root dry weight, mineral fertilization resulted as higher, organic fertilization intermediate and lower without fertilization, independently of the remaining evaluated factors (Figure 1c).

As for the percentage of root colonization by the AMF, there was only a significant difference with the factor Fertilization, which was higher in the treatments without fertilizer and organic fertilizer compared with mineral fertilizer (Figure 2).

The performance of the larvae in terms of biomass was different with respect to the different types of fertilization: highest with mineral fertilization, intermediate with organic fertilization and lowest without fertilization (Figure 3).

Experiment two

For the shoot dry weight, significant effects for all factors were obtained, and the “Sterilization x Cultivar” interaction was also significant (Table 1). Similarly, for root dry weight, significant effects for all the factors were observed with the exception of the Cultivar factor (Table 1). For the AMF root colonization, only a significant effect for the Insect factor was observed (Table 1), while for the larval weight a significant effect in the Fertilizer factor (Table 1) was observed.

The shoot dry weight was higher in plants with mineral fertilization than organic fertilization (Figure 4a) and lower with *S. frugiperda* compared to plants without *S. frugiperda* (Figure 4b). In general, soil sterilization caused an increase in the shoot dry weight, but with a greater response in the DK-2061 maize genotype compared with Criollo Odilon (Figure 4c).

In addition, higher root dry weight with mineral fertilization than organic fertilization (Figure 4d) was found and also lower root dry weight with *S. frugiperda* than without *S. frugiperda* (Figure 4e). Finally root dry weight was higher after soil sterilization as compared to unsterilized soil (Figure 4f).

For the percentage of root colonization by the AMF, there was only a significant difference with the Insect factor with higher colonization in plants with *S. frugiperda* compared to plants without *S. frugiperda* (Figure 5).

Regarding the larval weight variable, a significant difference was found only in the Cultivar factor in which the larval weight was higher in the Criollo Odilon genotype compared with the DK-2061 genotype (Figure 6).

4. Discussion

The main results of this work show that herbivory by *S. frugiperda* in maize was not affected by any of the studied factors such as fertilization type and soil sterilization rejecting the principal hypothesis that organic fertilization and the soil's microbiota reduce the damage caused by *S. frugiperda.*

Soil sterilization eradicated the native microorganism populations like the AMF and other beneficial fungi, such as *Trichoderma* that can change the phenotype of the plant by inducing resistance between masticating herbivores like *S. frugiperda* (Heinen et al, 2018; Contreras-Cornejo et al, 2018). Nevertheless, that was not the case in the present work. Similarly, AMF can change the nutrient content of foliar tissue, mainly P and N (Ravnskov and Larsen, 2016). In another similar work from the present doctoral project about maize-mycorrhiza-*S. exigua* interactions, it was found that a decrease of C/N in the maize's foliar tissue induced by AMF resulted in a higher biomass of the *S. exigua* larvae (Aguilar et al, Chapter 4). In the present experiment, the biomass of the larvae was not affected by the soil sterilization probably because that it did not cause substantial changes in the nutritional quality of the foliar tissue. Nevertheless, measuring the C, N and P is necessary to reinforce this theory. Differences in environmental factors from the soil and its associated biota in the mentioned experiments may explain the contrasting results.

On the other hand, the mineral and organic fertilization resulted in larvae with higher biomass than with the plants without fertilizers. The level of herbivory was seen to be reflected in the larval biomass, the highest maize growth was obtained with the mineral fertilization, followed by the organic fertilization and, lastly, the control without fertilization. Thus, the difference in the biomass of the larvae seems to be more a question of the abundance of foliar tissue and not its nutritional quality. Nevertheless, the results suggest that maize leaves of the Criollo Odilon genotype have better nutritional quality than leaves of the DK-2061 genotype. With the variables measured in this work, data is missing that can explain this observed effect, but it can be related to the nutrient profile like C, N and P, but most likely also with the defensive bioactive compound content.

Surprisingly, it was found that in the experiment with a high level of herbivory that there was no effect on the resource allocation to the roots and AMF, since the herbivory caused by the fall armyworm almost completely eliminated the foliar part of the plant, without a reduction in the resource allocation for the roots being noted. Conversely, for experiment two with a moderate level of herbivory, a reduction in root dry weight was shown, coinciding with a greater percentage of root colonization by the AMF. In general, the results of the two experiments show that herbivory by *S. frugiperda* in maize under experimental conditions does not affect the development of the mycorrhizal relationship.

The mineral fertilization reduced the root colonization by the AMF, which is a result that coincides with that found by Thomson et al (1992), reporting that mineral fertilization, especially with phosphorus, reduces the formation of symbiosis from arbuscular mycorrhizal fungi. On the other hand, even when it has been seen that the AMF developed better in organic matter (Albertsen et al 2006, Gosling et al 2006, Ravnskov et al 1999, Yu et al 2013), the organic fertilization that was used in the present study had no effect on root colonization by the AMF.

The maize plants grew more in the sterile soil than in the unsterile soil, but we also found greater plant biomass in the hybrid genotype than in the native. This is a documented response in maize, especially in the vegetative phase because of the energy input by the microbiota that absorbs the carbohydrates from the roots, such as the AMF and other endophyte microbes that inhabit the rhizosphere (Lopez-Carmona et al, 2019).

As the main conclusion of the present work, it can be observed that the damage caused by herbivory through *S. frugiperda* was not affected by the type of mineral or organic fertilization nor soil sterilization.

Acknowledgements

Figure legends

Figure 1a. Plant performance measured in grams of aerial dry weight from the different fertilization treatments (Without fertilizer, organic and mineral), in the absence and presence of

S. frugiperda, from an $n=6$, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

Figure 1b. Plant performance measured in grams of aerial dry weight from the two maize cultivars (Criollo Odilón and DK-2061), from an $n=6$, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

Figure 1c. Plant performance measured in grams of root dry weight from the distinct fertilization treatments (without fertilizer, organic and mineral), from an $n=6$, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

Figure 1d. Plant performance measured in grams of root dry weight from the two maize cultivars (Criollo Odilón and DK-2061). The averages are shown with the standard error measured from an $n=6$, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

Figure 2. Percentage of root colonization by arbuscular mycorrhizal fungi in the different fertilization treatments (Without fertilizer, organic and mineral). The averages are shown with the standard error measured from an $n=6$, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

Figure 3. Insect performance measured in grams of larval weight in the three fertilization treatments (Without fertilizer, organic and mineral). The averages are shown with the standard error measured from an $n=6$, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

Figure 4a. Plant performance measured in grams of aerial dry weight from the different fertilization treatments (Organic and mineral), from an *n*=6, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

Figure 4b. Plant performance measured in grams of aerial dry weight in the two insect treatments (absence and presence of *S. frugiperda*). The averages are shown with the standard error measured from an *n*=6, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

Figure 4c. Plant performance measured in grams of aerial dry weight from the two maize cultivars (Criollo Odilón and DK-2061), in the two sterilization treatments (disinfected and not disinfected). The averages are shown with the standard error measured from an *n*=6, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

Figure 4d. Plant performance measured in grams of root dry weight (PSR) in the two fertilization treatments (Organic and mineral). The averages are shown with the standard error measured from an *n*=6, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

Figure 4e. Plant performance measured in grams of root dry weight in the two insect treatments (absence and presence of *S. frugiperda*). The averages are shown with the standard error measured from an *n*=6, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

Figure 4f. Plant performance measured in grams of root dry weight in the two sterilization treatments (disinfected and not disinfected). The averages are shown with the standard error measured from an n=6, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

Figure 5. Percentage of root colonization by arbuscular mycorrhizal fungi (AMF) in the two insect treatments (absence and presence of *S. frugiperda*). The averages are shown with the standard error measured from an n=6, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

Figure 6. Insect performance measured in grams of larval weight in the two cultivars (Criollo Odilón and DK-2061). The averages are shown with the standard error measured from an n=6, analyzed with an LSD test with $p \leq 0.05\%$. The bars with a different letter vary significantly.

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Figures:

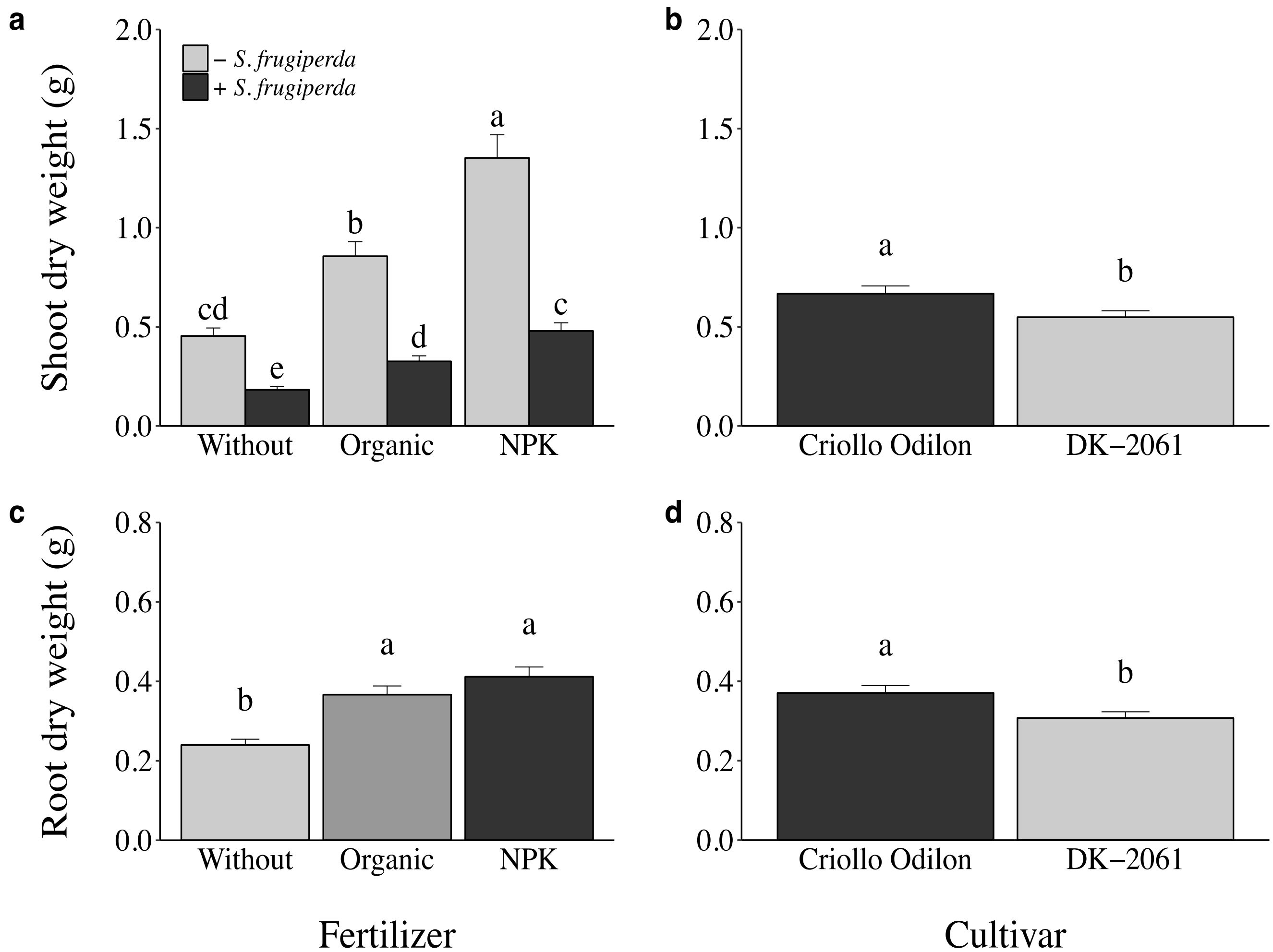
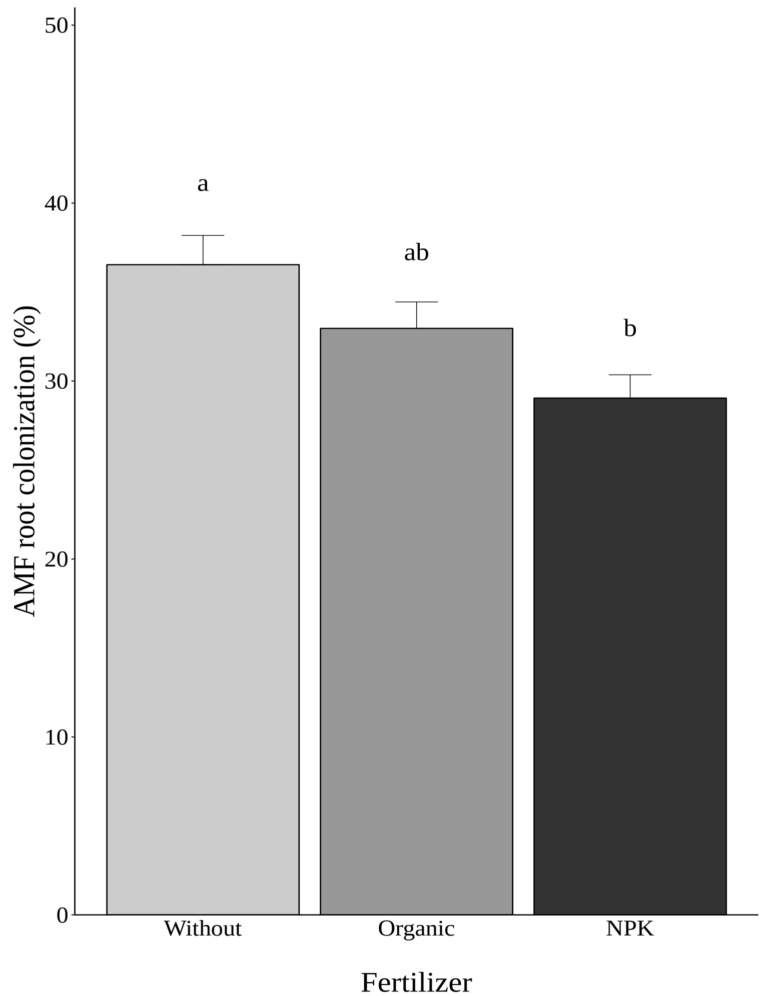


Figure 1. a), b), c), d).



AMF root colonization (%)
50
40
30
20
10
0
a
ab
b
Without
Organic
NPK
Fertilizer

Figure 2.

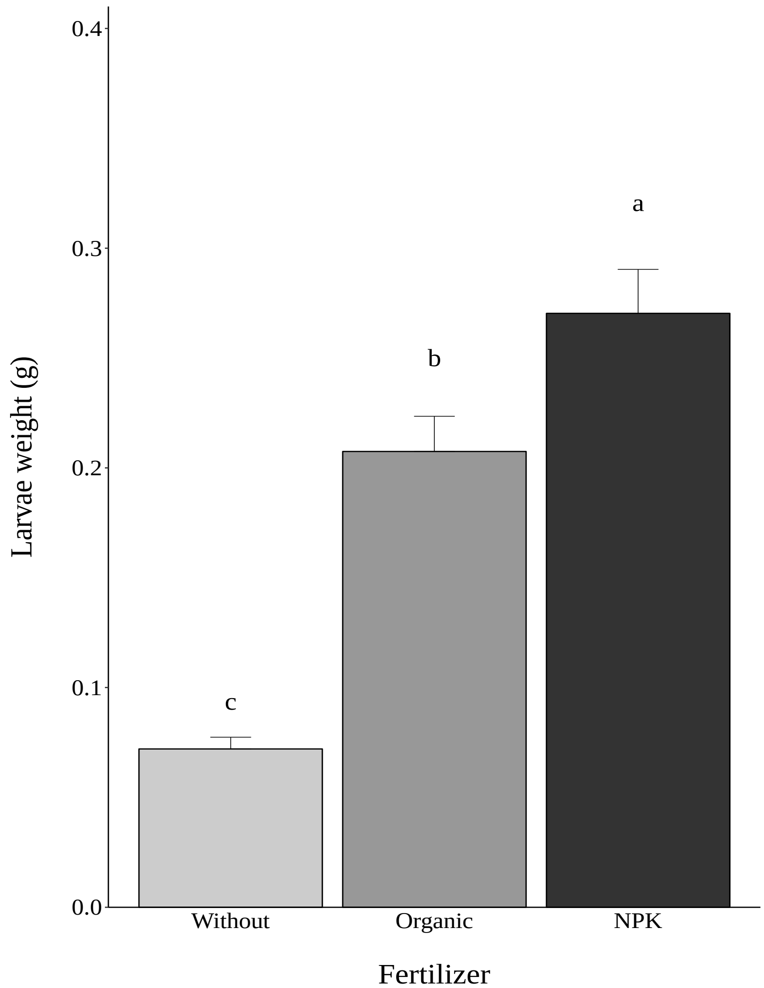

0.4
0.3
0.2
0.1
0.0
Larvae weight (g)
a
b
c
Without
Organic
NPK
Fertilizer

Figure 3.

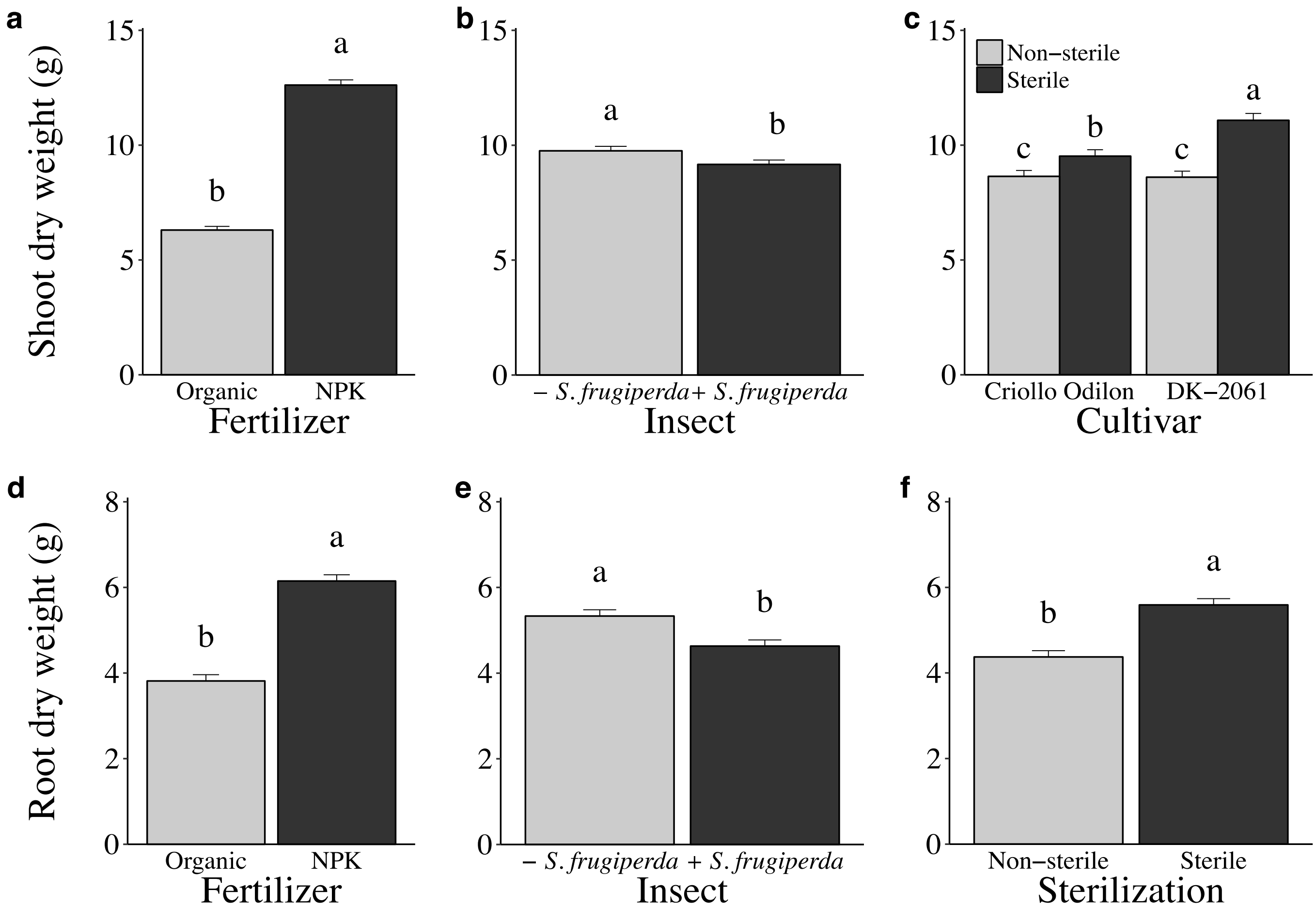
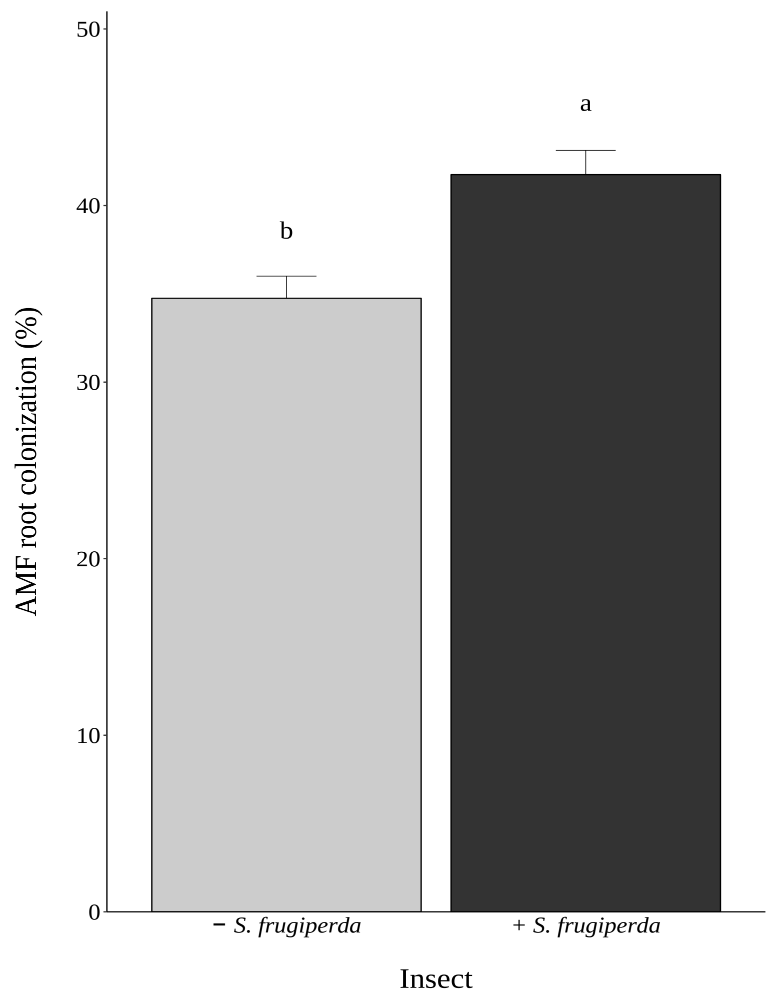
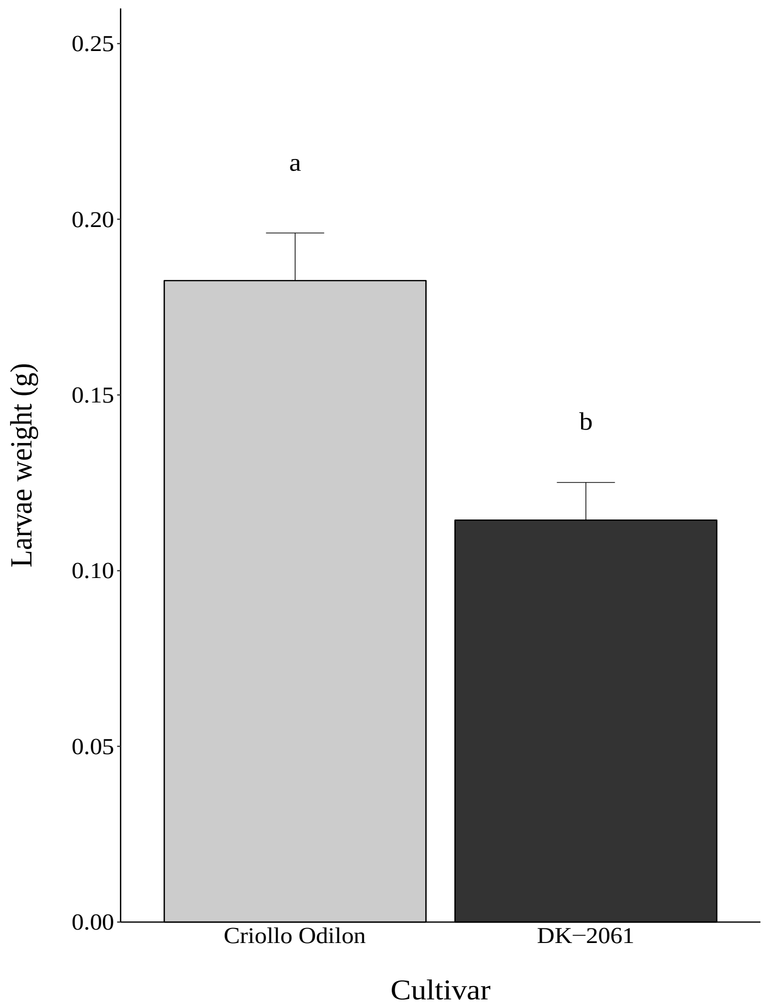


Figure 4. a), b), c), d), e), f).



50
40
30
20
10
0
a
b
AMF root colonization (%)
− S. frugiperda
+ S. frugiperda
Insect

Figure 5.



0.25
0.20
0.15
0.10
0.05
0.00
Larvae weight (g)
a
b
Criollo Odilon
DK−2061
Cultivar

Figure 6.

Tables:

Table 1. The *p* values obtained from statistical analysis performed through the GLM (*Generalized Linear Model*) in the two experiments one and two, of the factors Fertilizer, Cultivar, Insect and Sterilization and their interactions with all the meaured variables: Shoot dry weight (SDW), root dry weight (RDW), percentage of colonization by arbuscular mycorrhizal fungi (AMF) and larval weight (WLarva). (n=6).

	Experiment 1				Experiment 2			
Factors \ Variables	SDW	RDW	AMF	WLarva	ADW	RDW	AMF	WLarva
Fertilizer	***	***	**	***	***	***		
Cultivar	**	**			**			***
Insect	***				*	***	***	
Sterilization					***	***		
Fertilizer X Insect	***							
Cultivar X Sterilization					**			

*, $p \leq 0.05$, **, $p \leq 0.01$, ***, $p \leq 0.001$

CAPÍTULO 5. Manuscrito 3.

Mycorrhiza induced reduction in maize shoot C/N ratio results in improved growth of the insect herbivore Spodoptera exigua

Aguilar R.[1,2], Pozo M.J.[3], Díaz T[1], Azcón C.[3], Rivero J.[3], Punt M.[4], Larsen J[1]*

[1]Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México. Antigua Carretera a Pátzcuaro 8701, Col. Ex Hacienda San José de la Huerta, 58190. Morelia, Michoacán. México.

[2]Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo. Gral. Francisco J. Múgica S/N, Ciudad Universitaria, 58030. Morelia, Michoacán. México.

[3]Departamento de Microbiología del Suelo y Sistemas Simbióticos. Estación Experimental del Zaidín, (CSIC), Prof. Albareda 1, 18008. Granada, España.

[4]Departamento de Biología, Universidad de Utrecht. Heidelberglaan 8, 3584 CS Utrecht. Países Bajos.

*correo electrónico: *jlarsen@cieco.unam.mx*

Abstract

We performed a greenhouse pot experiment to investigate maize-mycorrhiza-insect multitrophic interactions. Hybrid and landrace maize were grown in sterilized low P soil without mycorrhiza and with single inoculation with the arbuscular mycorrhizal fungi (AMF) *Funneliformis mosseae*, *Rhizophagus irregularis* and *Claroideoglomus etunicatum* with two different P fertilization levels (low and high). Herbivory and larval growth of the insect herbivore *Spodoptera exigua* were examined over a three-day-period with six weeks old plants both *in-vitro* using detached leaves and *in-vivo* using clip cages with confined maize leaf areas. In general inoculation with AMF caused maize plant growth promotion and reduction in the shoot C/N ratio. No effects of AMF were observed regarding herbivory, but all three AMF improved final larval biomass substantially independent of assay system though with stronger

and more uniform effects in the *in-vitro* assay with detached leaves. In conclusion, under the experimental conditions employed in the present study AMF improved maize leaf food quality for larval growth of *S. exigua*, which seems to be linked with AMF induced reduction in shoot C/N ratio.

1. Introduction

Maize is one of the world basic cereal crops for human consumption and animal fodder (Ranum et al, 2014). Insect pests such as the foliar herbivore *Spodoptera exigua* are among the most production limiting factors in maize causing serious yield losses all over the world (Xia-lin et al, 2011). Pest control is most often achieved by insecticide applications (Pimentel, 2009), which however have adverse environmental drawbacks for human health and contamination of water and soil ecosystems with serious non-target effects on beneficial insect biota like natural insect pest enemies and pollinators (Pimentel, 2005). Biological pest control with natural enemies including pathogens (Lacey et al, 2015), predators and parasitoids (Chailleux et al, 2013), offer a possibility to reduce the environmental hazards from insecticide application either alone or in combination with a rational use of insecticides in an integrated pest management system (Blanco et al, 2014). Besides the direct chemical and biological insect pest control measures it is important to consider multitrophic plant-microbe-insect interactions to achieve efficient pest control (Koricheva et al, 2009). Beneficial root associated microorganisms are of special importance because they are known to alter to host phenotype in terms of both primary and secondary metabolites, which may induce resistance or tolerance to insect pests and hence alter the performance of the insect pest (Pineda et al, 2010, van Dam and Heil, 2011, Van der Putten et al, 2001). Most crops including maize are forming root associations with arbuscular mycorrhizal fungi (AMF), which are known to provide key ecosystem services in agroecosystems (Gianinazzi et al, 2010), mainly in terms of crop nutrition and health (Larsen et al, 2014). AMF are known to promote growth of maize though depending on maize genotype (Sawers et al, 2017; Alvarado-Herrejon et al, 2019) and agricultural practise such as fertilization (Sarabia et al., 2017; Lopez-Carmona et al, 2019).

Besides host growth promotion, AMF are also known to alter host nutrient composition (Ravnskov and Larsen, 2016) and induce production of plant defence compounds such as alkaloids and hormones (Lopez-Raez et al, 2010). Such phenotypic changes in the host plant induced by AMF most likely affect the palatability of the shoot tissue for insect herbivores, but information on this matter is limited.

AMF have been shown to affect the performance of foliar insect herbivores and hence affecting host plant health (He et al., 2017). From a meta-analysis study Koricheva et al (2009), reported that arbuscular mycorrhizal associations improve the performance of generalist chewing insects, whereas reduced performance was reported for specialist chewing insects and phloem feeders. However, in most studies on AMF and foliar insect herbivores focus on plant defence induction (Jung et al, 2012, Pozo and Azcon-Aguilar 2007, Pozo et al 2010), whereas limited information on how AMF mediated changes in host plant nutrition affect foliar insect herbivores is limited (Van der Heijden et al, 2006).

For an efficient insect pest management in maize agroeocosystems it is necessary to improve our knowledge about how alterations in maize nutrient content induced by mycorrhizal fungi affect the performance of maize shoot insect herbivores. Hence the objective of the present study was to investigate the effect of associating maize with different mycorrhizal fungi on the performance of *S. exigua.* Our main hypothesis was that mycorrhiza induced phenotypic changes will alter its food quality and hence growth of *S. exigua*.

2. Materials and methods

Experimental design

The experiment had a fully factorial design with the factors AMF with four levels (without, *Funneliformis mosseae*, *Rhizophagus irregularis* and *Claroideoglomus etunicatum*), maize genotype with two levels (the landrace Tuxpeño and the hybrid DK-2061) and P fertilization with two levels (25% and 75% Hewitt P). In total the experiment had 16 treatments (4 x 2 x 2) each with six replicates giving a total of 96 experimental units.

Growth substrate

The growth substrate was a sterile soil-sand-vermiculite (1/1/1; v/v/v) mix. The origin of the soil was from the “Cortijo Peinado” field (Fuente Vaqueros, Granada, Spain, 37º13’N, 3º45’W). The soil was a silt loam (clay, 17.15%; sand, 34.35%; silt, 48.50%) with the following properties: pH 8.4, total nitrogen (1.52 g kg^{-1}), total phosphorous (0.59 g kg^{-1}), total organic C (10.67 g kg^{-1}).

Maize genotypes

The maize *Zea mays* L. genotypes (landrace Tuxpeño and the hybrid DK-2061) from México were used in the present study and provided by the Agroecology Laboratory from Universidad Nacional Autónoma de México. Before sowing the seeds were surface sterilized according to Martinez and Wang (2009).

AMF inoculum

Three species of AMF were used including *Funneliformis mosseae* (EEZ 82), *Rhizophagus irregularis* (EEZ 59) and *Claroideoglomus etunicatum* (EEZ 163). The AMF inoculum was based on trap plant propagation and consisted of dry growth substrate with root segments, spores and mycelium as well as the growth substrate.

Insects

Spodoptera exigua larvae of the L3 instar were used in the herbivore assay. The larvae were obtained from eggs provided by Dr. Salvador Herrero Sendra of the Biotechnological Control of Pest Laboratory of the Universidad de Valencia, Campus Burjassot in Valencia, España. The insect colony was reared at 25°C, relative humidity of 70% and a photoperiod of 16:8 h (light:dark), with artificial diet to Lepidoptera larvae (Bell and Joachim 1976).

Experimental set-up

Plastic pots (1.1 liter) were filled with 1 liter growth substrate with the AMF inoculum mixed into the substrate according to treatments. To reestablish the AMF inoculum associated microbiota in the treatments without AMF ten ml of a filtrate mix of the three AMF inocula was added to per pot. The AMF microbiota filtrate was made by agitating 5 g of AMF inoculum with 500 ml MilliQ water for 24 h., which were then passed through a nylon filter with 20 µm mesh size. The AMF filtrates were mixed and 10 ml filtrate was added to each pot without AMF inoculum. Now one surface sterilized maize seed was sown in each pot.

Environmental conditions

Plants were maintained in a growth chamber at Estación Experimental El Zaidín in Granada with 24/16°C as day and night temperature, 16/8 hours day/night setting and 70% relative humidity.

Watering and fertilization

Plants were watered three times per week. Twice a week plants were watered with 50 ml of a Long Ashton nutrient solution (Hewitt, 1966) with 25% and 75% of the standard P concentration and once a week pots were watered to saturation according to weight.

Harvest and analyses

Plants were harvested eight week after sowing. The shoot was separated from the root and the roots carefully washed under running tap water. Shoot and root fresh weights were measured and subsequent plant tissue were dried with -80°C nitrogen and stored in a -80°C freezer.

Carbon (C) and Nitrogen (N) content in the shoot was measured at the Ionomic Laboratory of Technical Services of the *Centro de Edafología y Biología Agraria del Segura* (CSIC), Murcia, Spain. Total C and N contents were determined using an Elemental Analyzer (LECO TRUSPEC CN).

Determination of AMF root colonization was performed with the intersection method according to Giovanttti and Mosse (1980) after staining roots with black ink according to (Vierheilig et al., 2005).

Herbivory assay

Larval growth on maize shoot was examined *in-vitro* in a Petri dish assay with detached leaves and *in-vivo* with clip-cages. In the *in-vitro* assay two *S. exigua* larvae L3 were placed in a Petri dish with detached maize leaves and left for five days to feed. During the assay biomass of the larvae were measured daily and also the corresponding amount of maize biomass consumed was measured daily. In the hybrid maize DK-2061 a larval feeding assay was also performed using clip cages were one *S. exigua* larvae was added to each clip cage. The clip Cage (BioQuip) is a frame-less clip cage used to contain small insects on host plants. It is constructed of 81 x 81 mesh no-thrips screen bonded to two foam rings. The foam rings are placed on each side of the subject leaf. They are held in place by three splayed staples that are inserted in the appropriate positions of each ring. The angled shape of the staple serves to clamp the rings snugly against the leaf. Four staples are supplied with each cage. Each ring has an outside dimension of 1-7/16", inside dimension of 1" and thickness of 3/8" (36.5 x 25.4 x 9.5 mm).

Statistical analyses

All variables were subjected to statistical analyses with GLM fitted with lowest AIC exhaustive analysis. Treatment means were compared with post-hoc Tukey test with the least square means package. All statistical analyses were conducted using the “R” software version 2.9.2 (R Development Core Team).

3. Results

Shoot fresh weight

For shoot fresh weight significant single factor effects were observed (Table 1). Also significant “Maize genotype x P fertilization” and “Maize genotype x AMF” interactions were obtained (Table 1). While the landrace maize had higher shoot fresh weight with 75% P than with 25% P, no difference was observed for the hybrid maize (Figure 1a). On the other hand, though inoculation with all three AMF species promoted shoot fresh weight in both maize genotypes this effect was stronger in the hybrid maize (Figure 1b).

Root fresh weight

A significant three-way "Maize genotype x P fertilization x AMF" interaction was obtained for root fresh weight (Table 1). In the hybrid maize neither P fertilization nor AMF inoculation affected root fresh weight. On the contrary both these factors strongly affected root fresh weight in the landrace maize, where a differential response to AMF inoculation was observed with 25% P and 75% P was observed. In landrace maize AMF inoculation in combination with 25% had no effect or increased root fresh weight, whereas inoculation with any of the three AMF species in combination with 75% P fertilization reduced root fresh weight (Figure 2).

AMF root colonization

In terms of AMF root colonization only a significant single factor effect was observed for the factor AMF (Table 1), with a higher AMF root colonization obtained with *C. etunicatum* (% SE) than with the two other AMF *F. mosseae* (% SE) and *R. irregularis* (% SE).

Shoot C and N content

For shoot N content significant single factor effects were observed for all factors and also a significant "Maize genotype x AMF" interaction was obtained (Table 1). In hybrid maize inoculation with any of the three AMF species increased shoot N compared with that of without AMF inoculation, whereas no effect of AMF inoculation was observed for the landrace maize (Figure 3a).

For shoot C content significant single factor effects were observed for Maize genotype and AMF and also a significant “Maize genotype x AMF” interaction was obtained (Table 1). In the hybrid maize inoculation with the AMF *F. mosseae* reduced shoot C content, whereas the two other AMF had no effect. On the other hand, in the landrace maize all three AMF species caused a reduction in shoot C content compared to that of plants without AMF (Figure 3b).

Shoot C/N ratio

For shoot C/N ratio significant single factor effects were observed for all factors and also a significant “Maize genotype x AMF” interaction was obtained (Table 1). Shoot C/N ratio was higher in landrace maize than in hybrid maize. In both maize genotypes inoculation with any of the three AMF reduced shoot C/N compared to that of plants without AMF inoculation, except for *R. irregularis*, which had no effect on shoot C/N ratio in the landrace maize (Figure 3c).

Larvae biomass gain (%)

In the assay with detached leaves significant single factor AMF effects were observed after 24, 48 and 72 hrs (Table 1), where larvae biomass gain was higher in AMF inoculated plants (Figure 4 a,b,c). A similar pattern was obtained in the clip-cage assay, though only significant after 48 hrs (Table 1, Figure 4 d,e,f). A significant “P fertilization x AMF” was obtained in the clip-cage assay after 72 hrs (Table 1). In treatments with 25% P fertilization no significant effects of AMF inoculation was observed, whereas in treatments with 75% P fertilization inoculation with *C. etunicatum* resulted in a higher larvae biomass gain compared to that of the treatment without AMF inoculation (Figure 5).

4. Discussion

Here we show that individual maize mycorrhizal associations with the AMF *F. mosseae*, *R. irregularis* and *C. etunicatum* all increased maize shoot growth and reduced shoot C/N ratio resulting in improved growth of the foliar herbivore *S. exigua*, confirming our main hypothesis.

The observed plant growth promotion by AMF is a common maize phenotypic response (Zitlalpopoca et al, 2017; Sarabia et al, 2018) though depending on maize genotype (Sawers et al., 2017).

Mycorrhizal associations are also known to alter the amount and composition of shoot nutrients (Ravnskov and Larsen, 2016), which may alter its food quality for foliar herbivores (Vanette and Hunter, 2009). In the present study inoculation with AMF respectively decreased and increased shoot C and N content resulting in a decrease in the shoot C/N ratio, which coincided with an increase in the growth of *S. exigua* larvae. These findings suggest that leaves of mycorrhizal maize had higher food quality for *S. exigua*, since also no difference in herbivory level was observed for plants with and without AMF. Hence feeding on the same amount of leaves resulted in higher larval biomass when feeding on leaves of mycorrhizal plants.

Mycorrhizal associations have been suggested to reduce level of herbivory and hence damage from generalist insect chewing insects, also resulting in reduced larval growth (Koricheva et al, 2009), which however was not observed in the present study, where AMF had no effect on herbivory, but on the contrary increased larval growth. These findings suggest that under the experimental conditions employed in the present study AMF did not induce production of plant defence compounds, which otherwise would have resulted in less herbivory and hence less larval growth (Vanette and Hunter, 2009). However, this theory needs to be further addressed with measurements of a possible induction of plant defence compounds by AMF according to Jung et al (2012).

The observed increase in larval growth of the insect herbivore *S. exigua* caused by AMF is important to consider when evaluating the role of AMF in crop health. There is a general consensus that AMF improve crop health against pests either by increasing host plant resistance and/or tolerance against pests (St. Arnaud and Vujanivic, 2007). However, our results show that in the case of *S. exigua* this may not be the case since larvae with a higher biomass as observed from feeding on leaves of mycorrhizal maize, most likely result in higher pest vigour, survival and offspring production leading to higher pest populations. However, in the present study effect of AMF on life cycle parameters of *S. exigua* was not measured, which is needed for a more thorough discussion on this matter.

In conclusion, under the experimental conditions employed in the present study AMF improved maize leaf food quality for larval growth of *S. exigua*, which seems to be linked with AMF induced reduction in shoot C/N ratio.

Acknowledgements

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Figure legends

Figure 1. Shoot dry weight treatment means of 8-week-old maize plants of the interaction "Fertilization x Cultivar" with two levels of fertilization (25 and 75% P) and two maize cultivars (Landrace and hybrid) (a) and shoot dry weight treatment means of the "AMF x Cultivar" interaction with the AMF treatments (without, *F. mosseae*, *R. irregularis* and *C. etunicatum*) and the two maize genotypes (Landrace and hybrid) (b). Treatments with different letters are significantly different.

Figure 2. Root dry weight of 8-week-old maize plants of two maize genotypes (Landrace and hybrid) inoculated individually with *F. mosseae*, *R. irregularis* and *C. etunicatum* or left uninoculated and fertilized with 25% or 75% P. Treatments with different letters are significantly different.

Figure 3. Factor treatment means of AMF root colonization of 8-week-old maize plants individually inoculated with *F. mosseae*, *R. irregularis* or *C. etunicatum*. Treatments with different letters are significantly different.

Figure 4. Shoot N (a) and C(b) content and C/N (c) of 8-week-old maize plants of the treatment means from "AMF x Cultivar" interaction with the AMF treatments (without, *F. mosseae*, *R. irregularis* and *C. etunicatum*) and the two maize genotypes (Landrace and hybrid) (b). Treatments with different letters are significantly different.

Figure 5. Factor treatment means of larvae biomass gain from feeding on 6-week-old maize leaves from plants without AMF or with *F. mosseae*, *R. irregularis* and *C. etunicatum* with detached maize leaves (a, c, e) or with clip cages (b, d, f) after feeding for 24 hrs (a, b), 48 hrs (c, d) and 78 hrs (e, f). Treatments with different letters are significantly different.

Figure 6. Factor treatment means of larvae biomass gain from feeding for 72 hrs on maize leaves in clip cages of the "AMF x Fertilization" interaction with the AMF treatments (without, *F. mosseae*, *R. irregularis* and *C. etunicatum*) and two P fertilization levels (25 and 75% P). Treatments with different letters are significantly different.

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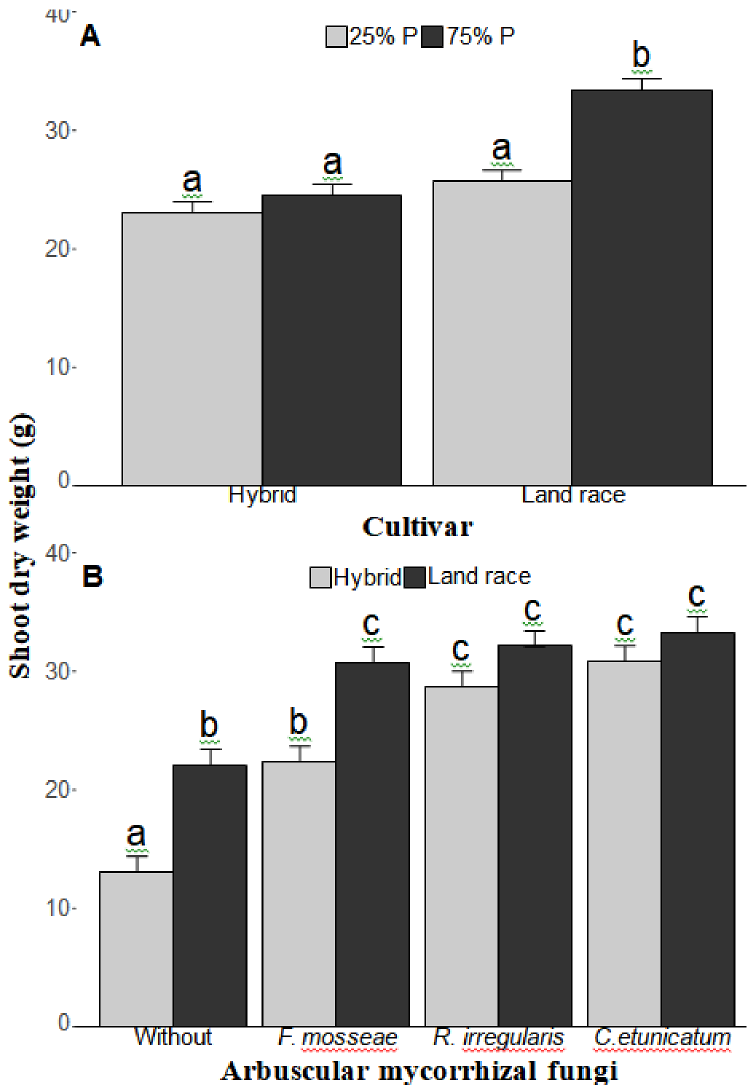

A
25% P
75% P
B
Hybrid
Land race
Shoot dry weight (g)
40
30
20
10
0
a
a
a
b
Hybrid
Land race
Cultivar
a
b
b
c
c
c
c
c
Without
F. mosseae
R. irregularis
C.etunicatum
Arbuscular mycorrhizal fungi

Figure 1. a),b).

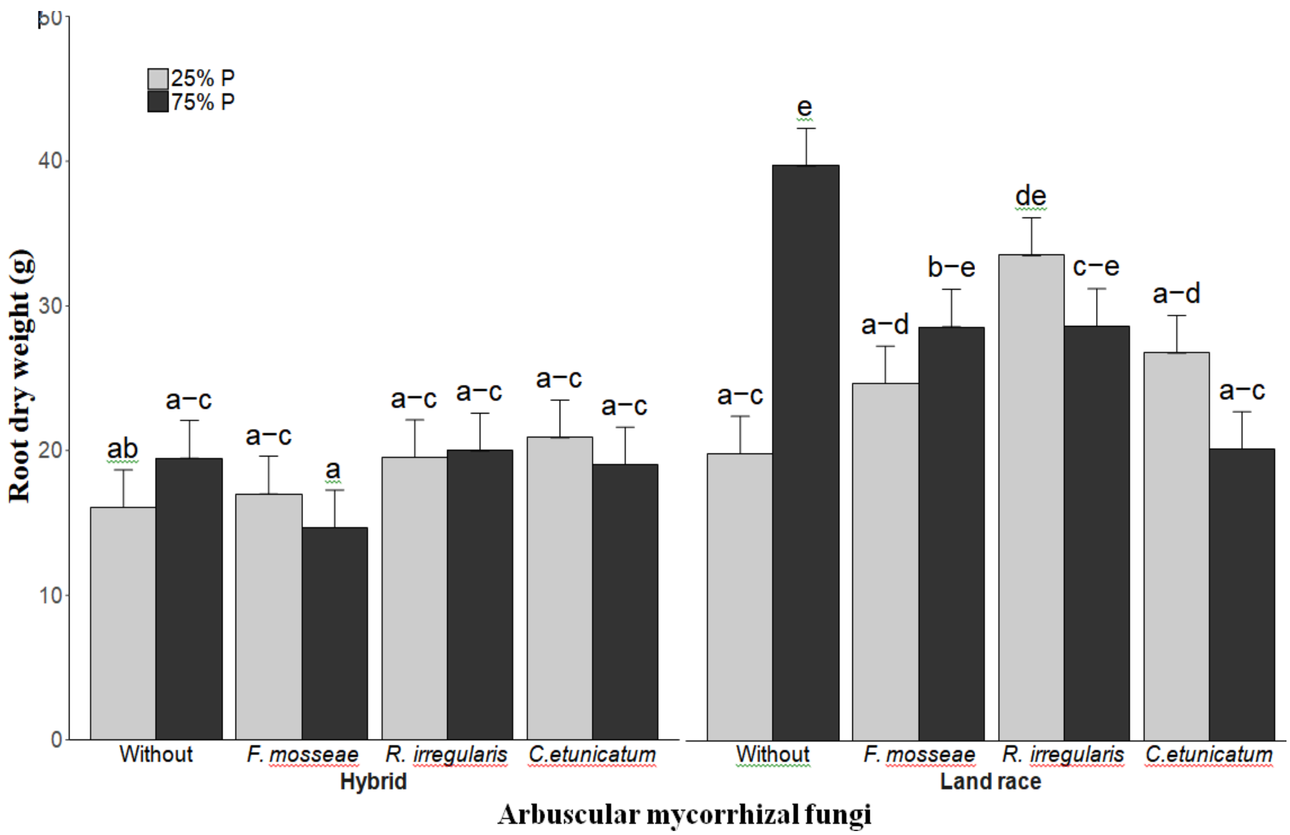
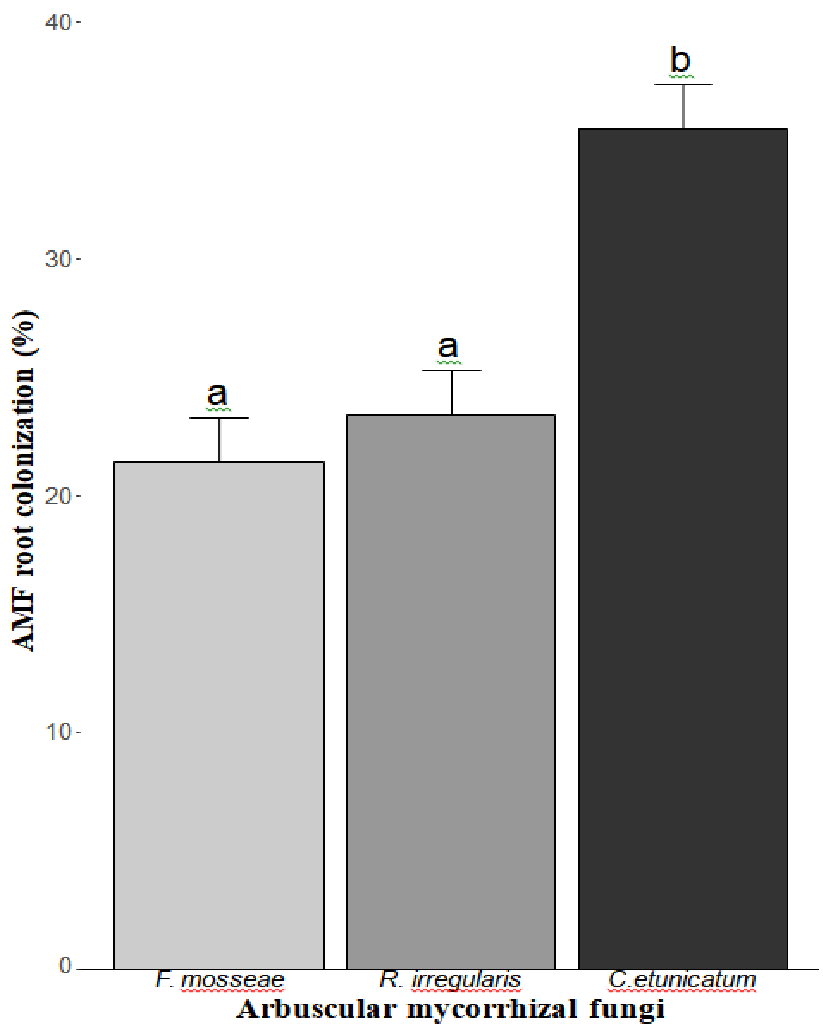

25% P
75% P
Root dry weight (g)
0
10
20
30
40
ab
a−c
a−c
a
a−c
a−c
a−c
a−c
a−c
e
a−d
b−e
de
c−e
a−d
a−c
Without
F. mosseae
R. irregularis
C.etunicatum
Hybrid
Without
F. mosseae
R. irregularis
C.etunicatum
Land race
Arbuscular mycorrhizal fungi

Figure 2.



40
30
20
10
0
AMF root colonization (%)
a
a
b
F. mosseae
R. irregularis
C.etunicatum
Arbuscular mycorrhizal fungi

Figure 3.

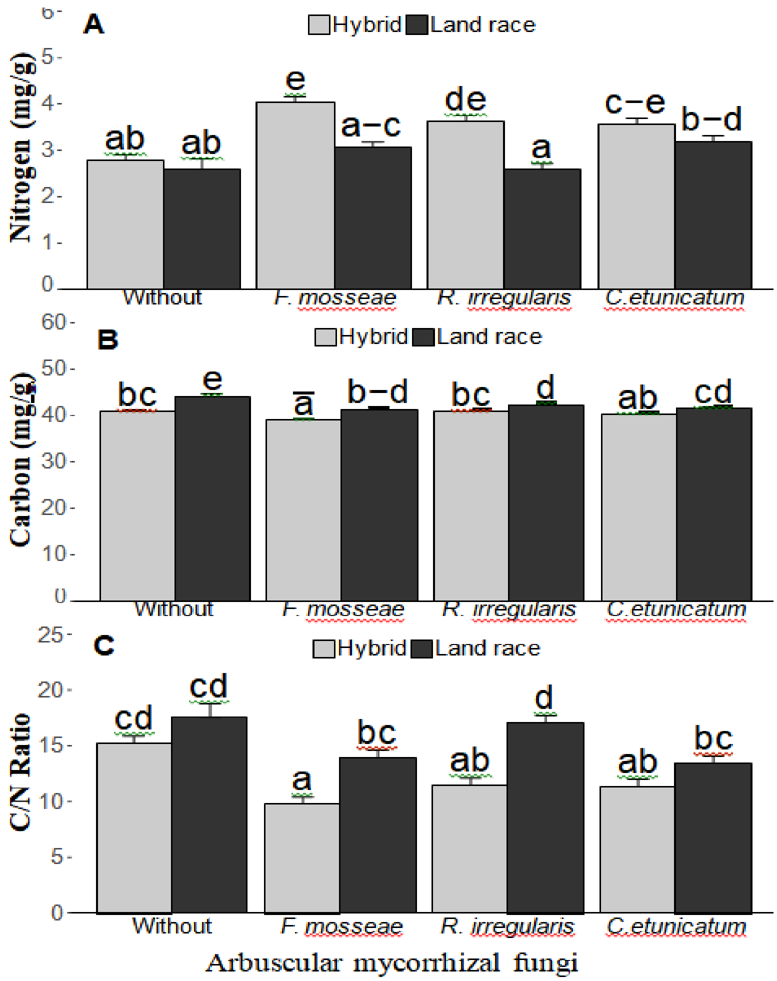

A
Hybrid
Land race
Nitrogen (mg/g)
ab
ab
e
a−c
de
a
c−e
b−d
Without
F. mosseae
R. irregularis
C.etunicatum
B
Hybrid
Land race
Carbon (mg/g)
bc
e
a
b−d
bc
d
ab
cd
Without
F. mosseae
R. irregularis
C.etunicatum
C
Hybrid
Land race
C/N Ratio
cd
cd
a
bc
ab
d
ab
bc
Without
F. mosseae
R. irregularis
C.etunicatum
Arbuscular mycorrhizal fungi

Figure 4. a),b),c).

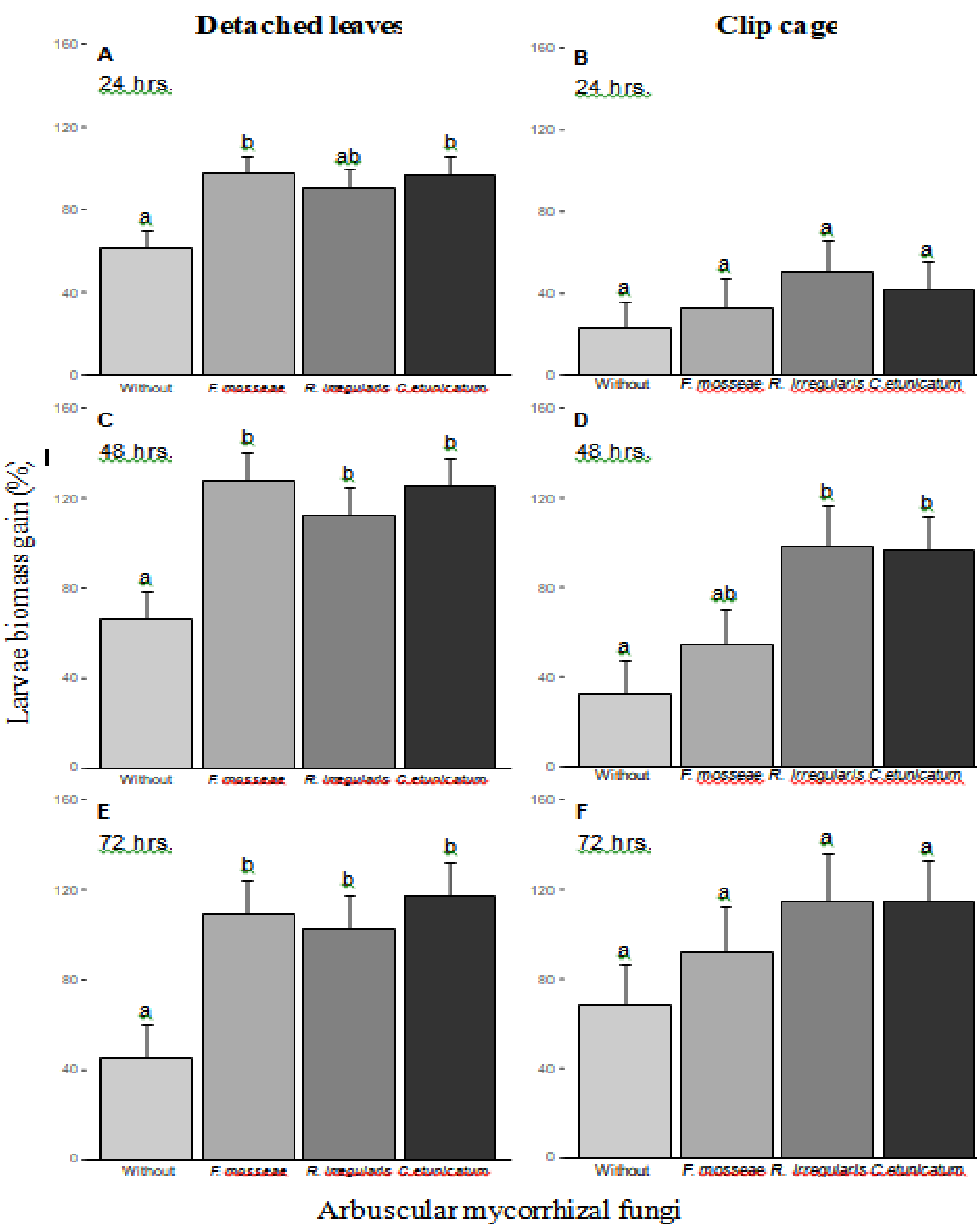


Figure 5. a),b),c),d),e),f).

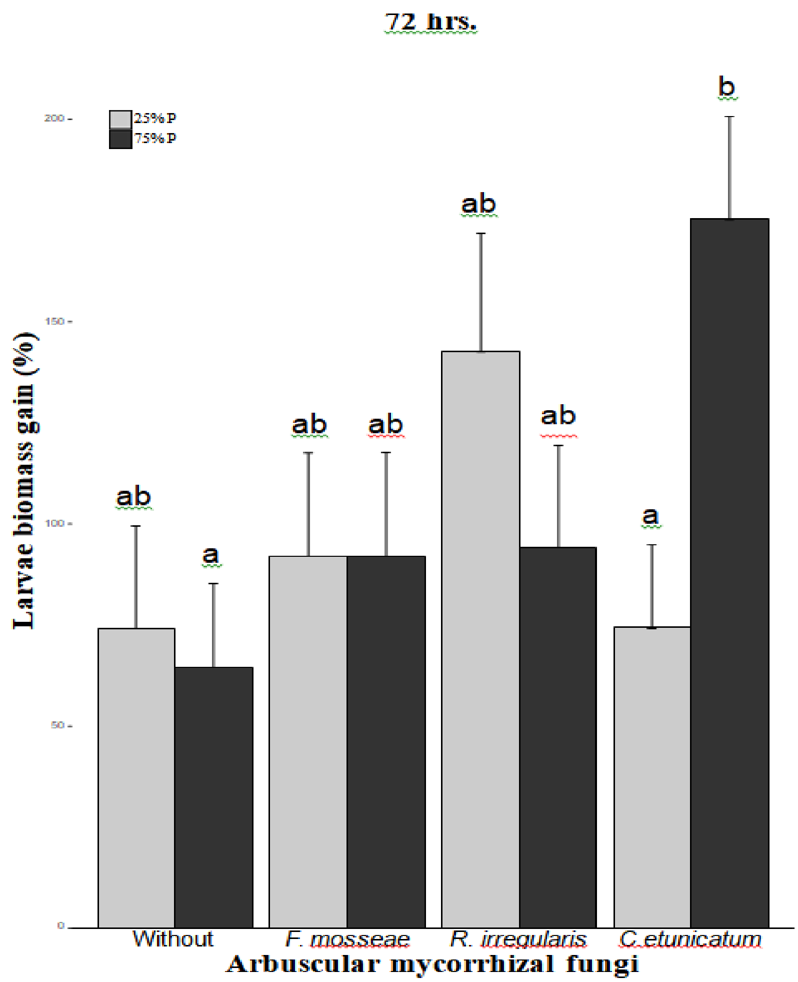

72 hrs.
25% P
75% P
200
150
100
50
0
Larvae biomass gain (%)
ab
a
ab
ab
ab
ab
a
b
Without
F. mosseae
R. irregularis
C.etunicatum
Arbuscular mycorrhizal fungi

Figure 6.

CAPÍTULO 6. DISCUSIÓN Y CONCLUSIONES

DISCUSIÓN

Para llevar a cabo un Manejo Agroecológico de Plagas y Enfermedades (MAPE) adecuado en el cultivo del maíz, es necesario conocer con más detalle los componentes e interacciones arriba-abajo del suelo involucrados (Altieri et al. 2012), con el fin de apoyar la búsqueda de nuevos conocimientos que nos ayuden a comprender estas interacciones (Wardle et al. 2004). Algunos de los temas que se evaluaron en este trabajo de investigación incluyeron: la influencia de la aplicación de fertilizantes orgánicos y minerales en la composición de microorganismos patógenos y de hongos micorrízicos arbusculares (HMA) en raíces de plantas de maíz (Gryndler et al. 2006), el crecimiento vegetal y la formación de micorrizas en respuesta a la herbivoría del gusano cogollero (*S. frugiperda*) en maíz y las interacciones multitróficas entre plantas de maíz, hongos micorrízicos arbusculares y el gusano soldado (*S. exigua*) (Barber et al. 2013, Barto et al. 2010)

Fertilización y cultivares de maíz

En general, la fertilización orgánica y mineral afectó positivamente los cultivares de maíz, obteniéndose el mayor crecimiento de plantas de maíz con la fertilización mineral, seguido de la fertilización orgánica y por último el testigo sin fertilizante. Para el caso de nuestro estudio (Capítulo 3), encontramos que la respuesta de los cuatro cultivares, dos nativos (Elotes Occidentales y Criollo Odilón) y dos híbridos (DK-2061 y DK-2062) fue parecida para casi todos los tratamientos: control sin fertilizante, fertilizante mineral (NPK), tres abonos verdes (avena, canola y janamargo), estiércol de res, composta y vermicomposta. En cuanto la ganancia de biomasa vegetal, después del control sin fertilizante, le siguieron los tres abonos verdes, avena, canola y janamargo, los tres que siguieron en ganancia de biomasa vegetal fueron el estiércol de res, la composta y la vermicomposta, en ese orden, por último, con el fertilizante mineral se obtuvo la mayor biomasa vegetal, tanto en peso seco parte aérea (PSPA), como en peso seco parte radical (PSPR).

Estos datos sobre el desempeño de los cultivares de maíz bajo distintos escenarios de fertilización, son importantes para el manejo de los agroecosistemas, ya que se conoce que algunos de los microorganismos que habitan en la rizósfera del maíz como los HMA y los patógenos de raíz, están relacionados con la salud y nutrición vegetal (Gianinazzi et al. 2010), además, se sabe también que responden a algunos de los componentes agrícolas como el tipo de cultivar, la labranza, la aplicación de plaguicidas y la fertilización (Larsen et al. 2015, Sawers et al. 2017).

Fertilización y microorganismos del suelo (HMA y patógenos)

Los cuatro microorganismos del suelo que fueron estudiados en esta parte del proyecto (poblaciones de campo nativas de HMA y los tres microorganismos patógenos de suelo *Pythium*, *Polymyxa* y *Microdochium*) presentaron respuestas diferentes hacia los tipos de cultivares de maíz y a los tipos de fertilización (Capítulo 3).

Fertilización orgánica y HMA

Para el caso de los HMA se encontró que la mayoría de los tratamientos de fertilización orgánica no tuvieron efectos significativos en cuanto al porcentaje de colonización. Aún y cuando se conoce que los HMA se desarrollan bien en la materia orgánica (Ravnskov et al. 1999; Albertsen et al. 2006; Gosling et al. 2006; Noble y Coventry 2005; Yu et al. 2013), los fertilizantes orgánicos empleados en este estudio (Capítulos 3 y 4), no mostraron efectos sobre la colonización radical excepto el abono verde canola, el cual causó una disminución en la colonización radical por HMA. La razón de estos resultados puede deberse a las diferencias en el tipo de materia orgánica examinada, relacionado con la dinámica de C, N y P en el suelo, limitando el crecimiento de los HMA (Larsen et al. 2015).

Fertilización mineral y HMA

En cuanto a la relación de la fertilización mineral con los HMA, encontramos que esta relación tuvo un comportamiento ambiguo (Capítulos 3 y 4). Se reconoce que la fertilización mineral, especialmente con aplicación de P, reduce la formación de simbiosis por parte de los HMA (Thompson et al. 1992), sin embargo, en el experimento del Capítulo 3, la fertilización mineral (NPK) no tuvo efecto sobre la colonización radical por HMA. Por otra parte, en nuestros resultados del experimento del Capítulo 4, encontramos que la fertilización mineral redujo la colonización de las raíces por HMA, resultado que coincide con lo encontrado por Thompson et al. 1992. Es probable que esta diferencia sea debido a que el suelo empleado estaba muy limitado en P para el desarrollo de la planta de maíz. En caso de una aplicación de P, esto permitiría un mejor desarrollo del hospedero y como consecuencia se distribuiría más C a la asociación micorrízica.

Además, encontramos que la colonización radical por HMA fue más alta en el maíz híbrido que en el nativo (Capítulo 3), lo cual contrasta con lo encontrado por Gavito y Varela (1993), pero en ambos casos, sólo unos pocos cultivares fueron incluidos, por lo que se sugiere que se hagan estudios con más cultivares de ambos maíces, esto, para mejorar el conocimiento ante la posibilidad de una pérdida de compatibilidad micorrízica del maíz híbrido comparada con el nativo. En trigo, se ha reportado pérdida de compatibilidad micorrízica para cultivares híbridos (Hetrick et al. 1992).

Fertilización orgánica y microorganismos patógenos

La fertilización orgánica afectó de manera diferente a los distintos tipos de microorganismos patógenos, donde encontramos que, en general, los microorganismos *Pythium* y *Polymyxa* fueron afectados negativamente, mientras que *Microdochium* fue afectado positivamente (Capítulo 3).

Se conoce que los abonos verdes reducen algunos patógenos de raíz, incluyendo nemátodos, oomycetes y hongos verdaderos, esto debido a la incorporación previa de algunos cultivos como avena, janamargo y algunas brasicáceas (Larkin 2013). En el presente estudio, esto se comprobó claramente (Capítulo 3), donde los tres tipos de abono verde (avena, canola y janamargo), suprimieron los niveles de infección de raíz por *Pythiun* y *Polymyxa*.

También se sabe que los patógenos de raíz son afectados negativamente por compostas o enmiendas que contienen porcentajes considerables de materia orgánica (Hoitink and Boehm 1999), lo cual se corrobora con los resultados obtenidos en el presente estudio (Capítulo 3.), donde se muestra que *Pythiun* y *Polymyxa* fueron suprimidos por todos los tipos de materia orgánica (estiércol de res, composta y vermicomposta). El modo de acción de la supresión por materia orgánica parece estar relacionada con el biocontrol que ejercen otros microorganismos por competencia de nutrientes y antibiosis (Hoitink y Boehm 1999).

En el caso del patógeno de raíz *Microdochium*, este se vio afectado positivamente por todos los tratamientos orgánicos, incluyendo los abonos verdes y las compostas.

La explicación de los efectos diferenciales entre estos microorganismos parece estar relacionada a su respectiva biología, los patógenos *Pythium* (hemi-biotrófico) y *Polymyxa* (biotrófico obligado) fueron reducidos por los fertilizantes orgánicos, mientras que *Microdochium* (saprótrofo facultativo) se vio incrementado por los fertilizantes orgánicos, hecho que concuerda con los resultados encontrados por Yu et al. (2012), donde mostraron que los hongos saprótrofos (como *Microdochium*) fueron más abundantes en raíces de plantas senescentes de chícharo, lugar donde había una mayor cantidad de materia orgánica en descomposición.

Fertilización mineral y microorganismos patógenos

En el caso de *Microdochium*, este patógeno se vio favorecido por todos los tratamientos orgánicos, solamente en el tratamiento con fertilización mineral este microrganismo no presento efectos, ya que siendo un hongo saprótofo, en este tratamiento, al parecer no había suficiente materia orgánica para su adecuado desarrollo.

Fertilización e insectos herbívoros foliares

Entre los principales resultados del Capítulo 4, encontramos que la herbivoría por *S. frugiperda* en maíz no fue afectada por ninguno de los factores estudiados, como el tipo de fertilización y la esterilización del suelo, rechazando la hipótesis principal de que la fertilización orgánica y la microbiota del suelo reducen el daño causado por *S. frugiperda.* Sin embargo, el nivel de herbivoría se vio reflejado en la biomasa larvaria, el mayor crecimiento del maíz se obtuvo con la fertilización mineral, seguido de la fertilización orgánica y por último, el control sin fertilización.

Estos mismos resultados se obtuvieron para la biomasa larvaria, mayor en fertilización mineral, seguida de la fertilización orgánica y las larvas con menor biomasa, de plantas sin fertilizar. La diferencia en biomasa de las larvas parece estar más relacionada a la cantidad de tejido foliar y no tanto a su calidad alimenticia. Sin embargo los resultados sugieren que las hojas de maíz del cultivar Criollo Odilón tienen mayor calidad alimenticia que las hojas del genotipo DK-2061. Con las variables medidas en este trabajo, faltan datos que puedan explicar este efecto observado, pero puede estar relacionado con el perfil de nutrientes como C, N y P y también con el contenido de compuestos bioactivos de defensa (Chen et al. 2008).

También se encontró que en el experimento con alto nivel de herbivoría (Capítulo 4), no hubo efecto de la asignación de recursos a las raíces y a los HMA, ya que, la herbivoría causada por el gusano cogollero eliminó casi por completo la parte foliar de la planta, sin notarse una reducción en la asignación de recursos para las raíces. En el caso de la otra parte del experimento, con nivel de herbivoría moderado se mostró una reducción en el peso seco de las raíces coincidiendo con un mayor porcentaje de colonización de las raíces por HMA. En términos generales, los resultados de los dos experimentos muestran que la herbivoría por *S. frugiperda* en maíz, bajo las condiciones experimentales descritas, no afecta el desempeño de las asociaciones micorrízicas.

Por otra parte, las plantas de maíz crecieron más en el suelo estéril que en el no estéril (Capítulo 4), además, encontramos mayor biomasa vegetal en el cultivar híbrido que en el nativo. Esto es una respuesta documentada en maíz, en especial en la fase vegetativa, por la inversión energética para la microbiota que absorbe los carbohidratos de las raíces como los HMA y otros microbios endófitos que habitan la rizósfera (Krauss et al. 2007).

Fertilización e interacciones bióticas microorganismo-planta-insecto

En otro trabajo similar del presente proyecto de doctorado (Capítulo 5), sobre las interacciones de maíz-micorriza-*S. exigua* se encontró que una disminución del C/N en el tejido foliar del maíz inducido por los HMA resultó en una mayor biomasa de las larvas de *S. exigua*. El incremento observado en el crecimiento larval del insecto herbívoro *S. exigua*, causado por los HMA es importante de considerar cuando evaluamos el papel de los HMA en la salud vegetal. Existe un consenso general de que los HMA mejoran la salud de las plantas contra las plagas, ya sea incrementando la resistencia y/o tolerancia de la planta hospedera contra las plagas. Sin embargo, nuestros resultados (Capítulo 5), muestran que en el caso de *S. exigua* esto no sucedió así, ya que larvas con mayor biomasa fueron alimentadas con hojas de maíz micorrizado resultando en insectos con mayor peso.

Las asociaciones micorrízicas son también conocidas por alterar la cantidad y composición de los nutrientes foliares (Ravnskov y Larsen 2016), lo cual también puede alterar la calidad alimenticia para los herbívoros foliares. En el presente estudio (Capítulo 5), la inoculación con HMA decreció e incrementó respectivamente el contenido de C y N foliar, resultando en un decremento en la relación C/N, lo cual coincide en un incremento en el crecimiento de la larva de *S. exigua*. Estos resultados sugieren que las hojas de maíz micorrizado tienen una mayor calidad alimenticia para

S. exigua, aunque también no se observaron diferencias en el nivel de herbivoría en plantas sin y con HMA. De ahí que, alimentarse sobre la misma cantidad de hojas resultó en una mayor biomasa larval cuando se alimentó con hojas de plantas micorrizadas.

Se ha sugerido que las asociaciones micorrízicas reducen el nivel de herbivoría y el daño por insectos masticadores generalistas, resultando también en una reducción del crecimiento larval (Koricheva et al. 2009), lo cual, sin embargo no fue observado en el presente estudio (Capítulo 5), donde los HMA no tuvieron efecto sobre la herbivoría, por el contario incrementaron el crecimiento larval. Estos resultados sugieren que bajo las condiciones experimentales empleadas en el presente estudio, los HMA no indujeron en la planta la producción de compuestos de defensa, lo cual, por el contrario, debió resultar en una menor herbivoría y por lo tanto en un menor crecimiento larval (Vannette y Hunter 2009). Sin embargo, esta teoría necesita ser tratada más a fondo con medidas de una posible inducción de compuestos de defensa de la planta por los HMA, de acuerdo a Jung et al. (2012).

CONCLUSIONES

La primera parte de este trabajo de investigación nos revela que el tipo de cultivar de maíz y la aplicación de fertilizantes orgánicos afectan la abundancia de las poblaciones de campo de HMA nativas y de los patógenos de raíz, en las plantas de maíz. En general, los fertilizantes orgánicos afectan diferencialmente a los diferentes grupos funcionales de hongos de raíz, lo cual, se ha visto, está relacionado con la biología del patógeno estudiado, esto es importante, ya que nos provee de información útil para un posible manejo de los HMA y los patógenos de suelo en los agroecosistemas de maíz.

También encontramos que la herbivoría por el gusano cogollero (*S. frugiperda*) en maíz no fue afectada por ninguno de los factores estudiados, como el tipo de fertilización y la esterilización del suelo, por lo tanto, rechazamos nuestra hipótesis de que la fertilización orgánica y los microorganismos del suelo reducen el daño causado por gusano cogollero. En el caso de la herbivoría total de la parte foliar por *S. frugiperda*, tanto el crecimiento de las raíces como la colonización de las raíces por HMA, no se vieron afectados, mientras que en el caso de la herbivoría moderada hubo mayor crecimiento foliar y mayor colonización de las raíces por HMA.

Además, se concluye que los tres tipos de hongos micorrízicos arbusculares (*F. mosseae*, *R. irregularis* and *C. etunicatum*) examinados aquí, mejoran la calidad alimenticia del maíz para el insecto herbívoro *Spodoptera exigua*, independientemente de la fertilización por fosforo. En general, todos los HMA incrementaron la parte foliar del maíz y redujeron la relación C/N, resultando en un crecimiento de las larvas de gusano soldado *S. exigua*.

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