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‘Mondragon’: A clonal plum rootstock to enhance management of Armillaria root disease in peach orchards of Mexico

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ABSTRACT

Root disease caused by *Armillaria mexicana* and/or *A. mellea* is associated with tree mortality in peach (*Prunus persica*) orchards within their primary production zone of the Mexican sub-tropics. To improve management options for this disease, the resistance/susceptibility reactions of three *Prunus* rootstocks to infection by *A. mexicana* (isolate MEX85) and *A. mellea* (isolate MEX100) were evaluated under greenhouse and field conditions. Greenhouse phase: *A. mexicana* and *A. mellea* were independently inoculated on 14 trees (10-month old) of each rootstock in polycarbonate containers: *P. persica* × *P. davidiana* ‘Nemaguard’, *P. persica* landrace ‘Criollos of La Goleta’ genotypes, and *P. salicina* Japanese plum ‘Mondragon’. Four non-inoculated trees of each rootstock were maintained as controls, and variables related to tree growth and disease incidence were monitored for all trees. At 22 months post-inoculation with *A. mexicana* in the greenhouse, ‘Mondragon’ showed the lowest (2.5%) incidence of infected roots (IRI), while ‘Criollos of La Goleta’ and ‘Nemaguard’ rootstocks showed the highest (21.6 and 24.6%, respectively) IRI. With greenhouse-grown plants inoculated with *A. mexicana*, the dry weights and volumes of roots, and dry weights of entire plants were greater (2.2–4.8 times higher) for ‘Criollos of La Goleta’; however, abundant mycelia were observed within the root collar and lateral roots of this rootstock. In contrast, *A. mellea* inoculation resulted in relatively low (17.5%) incidence of root infection. In a commercial orchard (field phase) with high (35.3% symptomatic and dead trees) incidence of *A. mexicana*, 21 plants of ‘Mondragon’ (propagated via stem cuttings), ‘Criollos of La Goleta’, and *P. mume* ‘Japanese apricot’ were inoculated with the *A. mexicana* isolate. In the field, ‘Mondragon’ showed the lowest (1.0 disease severity at 66 months post-inoculation, $P < 0.05$) susceptibility to *A. mexicana*. No *Armillaria* infection was found on control plants; however, *A. mexicana* and *A. mellea* were re-isolated from inoculated plants to fulfill Koch’s postulates. This work contributes to the development of efficient management strategies for *Armillaria* root disease in peach orchards in Mexico through the identification of ‘Mondragon’ as a rootstock with resistance to *A. mexicana*.

1. Introduction

Armillaria spp. are soil-borne root pathogens with a wide range of hosts, including important forest and fruit tree species, such as avocado (*Persea americana*), peach (*Prunus persica*), almond (*P. dulcis*), etc. (Baumgartner et al., 2011, 2018; Michua-Cedillo et al., 2017; Rivas--Valencia et al., 2017). In subtropical regions of Mexico, a high incidence of Armillaria root disease has been reported in several peach orchards (Coria-Avalos et al., 2005; Alvarado-Rosales, 2007; Fucikovsky, 2009; Elías-Román et al., 2013, 2018; Rivas-Valencia et al., 2017) within the primary production states, such as Morelos, Michoacan, and State of Mexico, that together account for 41.1% of the country’s domestic peach production (SIAP, 2017). In sites where peach orchards have been established in deforested areas, annual rate of peach tree mortality can

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exceed 90% (Fucikovsky, 2009; Michel-Aceves et al., 2015). This mortality is exacerbated by insufficient implementation of appropriate management strategies (Cox et al., 2005a).

The southern region of the State of Mexico is one of the leading peach-producing regions based on production volume and land area (Larqué-Saavedra et al., 2009; Rebollar-Rebollar et al., 2009; SIAP, 2017). In this region, a prominent limitation to peach production is tree mortality associated with Armillaria root disease, caused by *A. mellea*, *A. gallica*, and *A. mexicana*. The recently described species, *A. mexicana,* was found to have the highest distribution (66.6% of monitored orchards) and it was associated with 8.1–90.8% mortality of peach trees. In comparison, *A. mellea* was detected in 10.2% of monitored orchards and it was associated with 35.6% mortality of peach trees (Elías-Román et al., 2013; Michel-Aceves et al., 2015).

In commercial peach orchards of other Mexican states, such as Morelos and Michoacan, a single *Armillaria* species (reported as *Armillaria* sp.) has historically been assumed to be the cause of 21.3–99.9% mortality of peach trees (Fucikovsky, 2009; Rivas-Valencia and Fernández-Montes, 2017). Because virulence varies within the *Armillaria* genus (Cleary et al., 2012), it is necessary to evaluate the resistance or susceptibility of available commercial rootstocks (hosts) to virulent isolates of the *Armillaria* species that are prevalent within the production areas of Mexico.

In other countries where *Armillaria* spp. causes mortality of peach trees, use of resistant rootstocks is a recommended management strategy (Guillaumin et al., 1991; Beckman and Pusey, 2001; Cox et al., 2005a; Beckman, 2007). For example, rootstocks, such as 'Sharpe' and 'PM-29', have been reported by Beckman et al. (2012) to have resistance to *Desarmillaria tabescens* (as *A. tabescens*) (Koch et al., 2017), and rootstocks of Myrobalan plum (*P. cerasifera*) and its interspecific hybrids were found resistant to *A. mellea* and *D. tabescens* (Baumgartner et al., 2018). In addition, Japanese plum (*P. salicina*) and Japanese apricot (*P. mume*) were found to be resistant to *A. mellea* (Raabe, 2008).

Effective management strategies to reduce mortality of peach trees caused by *Armillaria* spp. are essential to maintain the longevity and profitability of commercial orchards in the most prominent peach-production areas of Mexico. For this purpose, the objective of this work was to evaluate the resistance/susceptibility of four *Prunus* rootstocks to infection by *A. mexicana* and *A. mellea* under greenhouse and/or field conditions.

2. Materials and methods

Two experiments were conducted with four peach rootstocks: *P. persica* × *P. davidiana* 'Nemaguard', *P. persica* landrace genotypes, *P. mume* seedlings Japanese apricot, and *P. salicina* Japanese plum 'Mondragon'.

2.1. Prunus rootstock trial in greenhouse

The first trial was performed from January 22, 2010 to November 21, 2011 (22 months) in a greenhouse [N19°27′39.37″, W98°54′15.44'']; 2400 masl; climate = C (w2)] located at College of Postgraduates, Montecillo, Texcoco, State of Mexico. The planting substrate was unsterilized loam soil (pH = 4.5, CE dS/m = 1.23, organic matter = 0.27%) from the commercial peach orchard called Mondragon orchard # 1.

2.2. Prunus rootstocks test in field conditions

The second test was implemented from November 6, 2009 to May 2015 (66 months after planting) in the 'Mondragon' orchard # 1 (Cruz de Piedra, Coatepec Harinas, State of Mexico; N18°56′41.1″, W99°48′52.3″, 2260 masl, C (w2) with 1500–2000 mm average annual rainfall), with trees displaying a high (35.3% in 2011) incidence of symptomatic trees with chlorotic foliage and gummosis at the trunk base; and tree mortality associated with *A. mexicana* (Elías-Román, 2013).

2.3. Isolation and inoculum preparation

Field inoculations were performed with *A. mexicana* (isolate MEX85) obtained from a dead peach tree in Mondragon orchard # 1 (Elías-Román et al., 2018). A basidiome-derived culture (MEX85 = CM-CNRG-433) was deposited in the microorganism collection (CM) at National Center of Genetic Resources (CNRG) – National Institute of Forestry, Agriculture and Livestock Research (CM-CNRG-INIFAP), Tepetitlan de Morelos, Jalisco, Mexico. The greenhouse experiment also included *A. mellea* sensu stricto (isolate MEX100), which was also collected from a peach tree adjacent to the collection site of MEX85 isolate in Mondragon orchard # 1 (Elías-Román et al., 2013).

Armillaria cultures were grown on malt-agar medium (3% malt extract, 3% dextrose, 1% peptone, 1.5% agar) in Petri dishes at 22 °C. Seeds (acorns) of oak (*Quercus* sp.) were used to increase *Armillaria* inoculum, according to the procedure of Beckman and Pusey (2001) with the following modifications. Insect-damaged or non-viable acorns were removed by flotation in water, while the other acorns remained submerged for 12 h. After soaking, 100 acorns were deposited in glass bottles (1 L) with 100 mL distilled water and sterilized at 1.03×10^5 Pa (15 psi) for 1 h. After 24 h, three discs (1.0 cm diameter) of 25-day-old mycelia of *A. mexicana* (MEX85) or *A. mellea* (MEX100) were deposited within each bottle. The bottles containing the acorns and the corresponding *Armillaria* isolate were incubated in continuous darkness at room temperature (ca. 20 °C). The inoculum was used after the acorns were completely colonized by the *Armillaria* sp. at ca. 2 months after the inoculation.

2.4. Experimental rootstocks

Three rootstocks were evaluated for greenhouse inoculations: *P. persica* × *P. davidiana* 'Nemaguard'; *P. persica* landrace 'Criollos of La Goleta' genotypes widely used in southern State of Mexico region (Acosta-Cruz et al., 1998); and *P. salicina* Japanese plum 'Mondragon'. The 'Nemaguard' trees were purchased from a commercial nursery in Coatepec Harinas, State of Mexico; the 'Criollos of La Goleta' trees came from La Goleta region (southern State of Mexico). 'Nemaguard' and 'Criollos of La Goleta' were propagated by seed, but 'Mondragon' was propagated by stem cuttings from basal shoots collected from a tree located (N19°20′54.15″, W98°11′19.65″) on private property, in Atzitzimititlan locality, municipality of Apetatitlan de Antonio Carvajal, Tlaxcala, Mexico. In that location, a single plant of the 'Mondragon' rootstock was used to maintain two different varieties of *P. persica* ('Cardenal' and 'Robin') for 7 years. In addition, vegetative compatibility with peach was further confirmed by grafting rooted cuttings of 'Mondragon' with *P. persica* 'Snow Tropic,' which resulted in favorable performance over 3 years. Clonal materials of 'Mondragon' (*P. salicina*; Japanese plum) have been deposited (tracking number CNRG: 2081800001) under *in vitro* culture conditions of minimal growth in the laboratory at National Center of Genetic Resources - National Institute of Forestry, Agriculture and Livestock Research (CNRG-INIFAP), Tepetitlan de Morelos, Jalisco, Mexico.

Three *Prunus* species were evaluated as rootstocks for field inoculations: *P. mume* 'Japanese apricot', which was propagated via seeds collected from a tree established in an experimental orchard (N19°27′30.63″, W98°54′15.75″, 2395 masl) at College of Postgraduates, Montecillo, Texcoco, State of Mexico, Mexico, as well as 'Criollos of La Goleta' propagated by seed, and 'Mondragon' propagated clonally. Prior to the initiation of both studies, trees were maintained in plastic containers (30 cm length x 15 cm width) for 7 months. Non-grafted plants were used for both field and greenhouse studies.

2.5. Rootstock inoculation with Armillaria spp

In the greenhouse, trees (10-month old) of each rootstock were transplanted on January 22, 2010 into three polycarbonate containers (crates): two of larger size (160 × 60 × 33 cm; length, width, and height, respectively), and a third of smaller size (80 × 60 × 33 cm). In the two large containers, 21 plants (seven replications of each rootstock) were inoculated independently with *A. mexicana* (MEX85) and *A. mellea* (MEX100) (Elías-Román et al., 2013, 2018), for a total of 42 plants inoculated with *Armillaria* spp. In the third crate, 12 non-inoculated trees served as controls; the plantation spacing was 15 × 20 cm. For the inoculation, two acorns individually colonized with *A. mexicana* or *A. mellea* isolates were placed in contact with the primary or secondary (lateral) roots (without causing wounds) of each tree, approximately 15 cm below ground level. The plants were irrigated daily for 30 min with stored rainwater, using an irrigation timer (Orbit® model 91600) connected to a drip irrigation system with drippers of 4 Lh^{-1}, each one with four injection stakes; one stake per tree.

In the field, experimental rootstocks (8-month old) were established in August 2009, approximately 40 cm apart from the base of *Armillaria*-infected symptomatic or dead trees. To reduce probability of rootstock escape from natural infection by pre-existing field inoculum, all planted trees were subsequently inoculated with two acorns colonized with *A. mexicana* at 2-months post-establishment. A metal bar was used to open a hole (3 × 15 cm, diameter × depth) adjacent (±2.0–4.0 cm) to the rootstock base, colonized-acorn inocula were deposited in the root zone, and the hole was filled with surrounding soil. During the dry season (December to May), furrow irrigation was applied to saturation point once per month. At the end of this study, root sections of *Armillaria*-inoculated and control trees were used for re-isolation of *Armillaria* spp. in BDS culture medium (Worrall, 1991) to fulfill Koch's postulates.

2.6. Variables evaluated

For the greenhouse study, rootstocks were removed from the containers at 22 months post-inoculation, and the following variables were recorded: Dead root (DR) incidence % (DRI) = [(Number of DR with signs of *Armillaria* in relation to the total number of primary and/or secondary (lateral) roots (PLR) per plant) x 100].

Infected root incidence % (IRI) = [(Number of live roots infected + DRI)/PLR × 100].

Root dry weight (RDW) was obtained by drying roots in perforated paper bags in a forced-air oven (Grieve, LW-201C) at 70 °C until a constant weight (g) was achieved (ca. 72 h).

Root length (RL), was estimated by summing the lengths (cm) of PLR for each plant.

Root volume (RV) was determined by measuring the volume (mL) of water displaced by immersing the roots in graduated cylinder (2 L) containing 1 L water.

Plant dry weight (PDW) was obtained by drying and weighing (g) the whole plant (roots, stem, and leaves) dried in perforated paper bags within a forced air oven at 70 °C for ca. 72 h.

To evaluate the field study, soil around the tree base was carefully excavated to expose the root collar and main lateral roots to a depth of ca. 30 cm. Symptoms and signs associated with *Armillaria* infection in the root crown and lateral roots were recorded. Subsequently, the excavated area was filled in again with soil to maintain the viability of the trees and to observe the development of the disease severity.

Severity of *Armillaria* infection was measured according to a five-class, disease-severity scale (Fig. 1), similar to that reported by Metaliaj et al. (2006), but with modified categories:

0 = Healthy tree, with no signs of *Armillaria* in roots.

Fig. 1. Armillaria disease severity scale of five classes (0–4) in *Prunus* spp. exposed to *Armillaria* in the field, where: 0 = Healthy tree, with no signs of *Armillaria* in roots. 1 = Tree without disease symptoms or dead roots, but with one or more primary or lateral roots with initial colonization by a mycelial fan of *Armillaria*. 2 (a-b) = Tree without disease symptoms in the foliage, presence or absence of gummosis in root crown and/or stem, and living primary or secondary root, colonized with subcortical mycelial fan of *Armillaria*, and presence or absence of *Armillaria* rhizomorphs (2a) dead primary or secondary root, colonized with subcortical mycelial fan of *Armillaria*, and presence or absence of rhizomorphs (2b). 3 (a-c) = Tree with or without above-ground disease symptoms (gummosis, chlorotic leaves), subcortical colonization by *Armillaria* mycelial fans in the root crown and/or one or more roots (3a and 3b), one or more dead roots infected by *Armillaria*, presence or absence of rhizomorphs (3c). 4 = Dead tree with at least one mycelial fan, presence or absence of *Armillaria* rhizomorphs in a primary/lateral root or root crown. Arrows show symptoms (gummosis in stem and production of new roots generated around the affect area) and signs (subcortical mycelial fan and presence of rhizomorphs) of *Armillaria*.

1 = Tree without disease symptoms or dead roots, but with one or more primary or lateral roots with initial colonization by a mycelial fan of *Armillaria*.
2 = Tree without disease symptoms in the foliage, presence or absence of gummosis in root crown and/or stem, and primary root or secondary dead roots with subcortical mycelial fan of *Armillaria*, and presence or absence of *Armillaria* rhizomorphs.
3 = Tree with or without above-ground disease symptoms (gummosis, chlorotic leaves), subcortical colonization of *Armillaria* mycelial fan in the root crown and/or, one or more dead roots, presence or absence of *Armillaria* rhizomorphs.
4 = Dead tree with at least one mycelial fan, presence or absence of *Armillaria* rhizomorphs in a primary/lateral root or root crown.

Based on *Armillaria* disease severity values for each experimental unit, we obtained a weighted average severity by rootstock, according to the formula of Guzmán-Plazola et al. (2004):

$$WAS = [4\ (Np4) + 3(Np3) + 2(Np2) + 1(Np1) + (Np0)] \div Ntp$$

Where: WAS (weighted average severity), Np4 = number of plants with disease severity of 4, Np3 = number of plants with disease severity of 3, Np2 = number of plants with disease severity of 2, Np1 = number of plants with disease severity of 1, Np0 = Number of plants with disease severity of 0, Ntp = Total number of plants evaluated in each treatment.

The WAS values were registered at 40, 59, and 66 months after inoculation (January 2013, October 2014, and May 2015, respectively).

2.7. Experimental design and statistical analysis

In the greenhouse, a split plot with completely randomized design was used with a factorial arrangement considering three rootstocks ('Nemaguard', 'Criollos of La Goleta', and 'Mondragon'), three types of inoculum (control, *A. mexicana*, and *A. mellea*), and seven and four repetitions per treatment (seven repetitions for combinations that included the *Armillaria* species inoculated and rootstocks and four repetitions for the non-inoculated control treatments and rootstocks), each tree served as an experimental unit. Before ANOVA analyses, incidence value percentages were transformed into their arcsin values using $\arcsin\sqrt{x/100}$. The retransformed means to original units are shown in Table 1 and are discussed in the text. The ANOVA was performed with the GLM procedure and the comparison of means with LSmeans (SAS®, 2011).

In the field, trees of the different rootstocks were established in a randomized block design, with seven replications, an individual tree served as an experimental unit. The severity values of field experiment were analyzed using the Friedman test and the multiple comparison of ranges means by default in InfoStat (2008).

3. Results

3.1. Susceptibility of rootstocks to Armillaria spp. in the greenhouse

At 22 months post-inoculation, the rootstocks showed differences ($P < 0.05$) in infected root incidence (IRI) and dead root incidence (DRI) by *Armillaria* spp. 'Mondragon' showed a high resistance to *A. mexicana* and *A. mellea*, evidenced by the lowest averages with 2.5% IRI and 0% DRI. 'Criollos of La Goleta' and 'Nemaguard' showed high susceptibility to *A. mexicana*, and *A. mellea*, with similar values (21.6 and 24.6 IRI; 7.2 and 9.2 DRI, respectively) (Table 1).

According to the IRI values, *A. mexicana* was more virulent, with IRI values of 31.2%, which was almost double the IRI (17.5%) obtained with *A. mellea* (Table 1). Infection by *A. mexicana* was characterized by larger subcortical mycelial fans (Fig. 1: 2b) and partial necrosis of the xylem (sapwood and/or heartwood) at the root crown level in the susceptible hosts, 'Nemaguard' and 'Criollos of La Goleta'; however, *A. mexicana* only caused mortality of one 'Nemaguard' tree at the end of the study period in 2012 (22 months post-inoculation). In contrast, *A. mexicana* root crown infection in the resistant 'Mondragon' rootstock was surrounded by abundant dark coloration and callusing of cortical and subcortical tissue, forming vertical lesions, and production of new roots generated around the affected area (Fig. 1: 3b). In comparison, the less virulent *A. mellea* only infected roots of 'Nemaguard' and 'Criollos of La Goleta' (Table 1). Plants of these genotypes showed no external symptoms of infection, such as chlorosis, dieback, and/or gummosis at root collar, although signs of *Armillaria* and necrosis in sapwood and heartwood were observed upon dissection of primary and secondary roots (Fig. 1: 1). The control (uninoculated) trees maintained healthy roots throughout the experiments. No interaction ($P > 0.05$) was observed among *Armillaria* isolates and rootstocks on the basis of IRI. However, interactions among *Armillaria* species and rootstock was apparent on the basis of DRI, therefore, the evaluated rootstocks performed differently

Table 1
Percentage of infected and dead roots in three rootstocks inoculated with either of two *Armillaria* species, and the *Armillaria* sp.-rootstock combinations at 22 months post-inoculation in the greenhouse (Texcoco, State of Mexico, Mexico).

Rootstock/*Armillaria* species inoculated		n[a]	Infected root incidence (IRI) %[b]	Dead root incidence (DRI) %[b]
'Nemaguard'[c]		18	24.6 a	9.2 a
'Criollos of La Goleta'[d]		18	21.6 a	7.2 a
'Mondragon'[e]		18	2.5 b	0.0 b
A. mexicana		21	31.2 a	13.1 a
A. mellea		21	17.5 b	3.3 b
Control		12	0.0 c	0.0 b
A. mexicana	'Nemaguard'	7	49.2 a	26.4 a
	'Criollo of La Goleta'	7	36.7 a	12.8 b
	'Mondragon'	7	7.6 b	0.0 c
A. mellea	'Nemaguard'	7	24.5 a	1.2 bc
	'Criollo of La Goleta'	7	27.9 a	8.7 bc
	'Mondragon''	7	0.0 b	0.0 c
Control	'Nemaguard	4	0.0 b	0.0 c
	'Criollo of La Goleta'	4	0.0 b	0.0 c
	'Mondragon'	4	0.0 b	0.0 c

[a] Replications.
[b] In each column, means followed by different letters are significantly different, based on LS-means ($P \leq 0.05$).
[c] *Prunus persica* × *P. davidiana* (Chinese wild peach).
[d] Landrace of *P. persica* (peach).
[e] *P. salicina* (Japanese plum).

with each *Armillaria* species (Table 1).

The *Armillaria* species used for inoculation produced no detectable differences for root dry weight (RDW), root volume (RV), root length (RL), and plant dry weight (PDW), but significant differences were noted among the rootstocks. The highest growth was observed in 'Criollos of La Goleta', followed by 'Nemaguard'. 'Mondragon' had the lowest values of RDW, RV, RL and PDW (Table 2). The 'Criollos of La Goleta' inoculated with *A. mexicana* obtained the highest means with significant differences based on RDW, RV, RL, and PDW, compared with the other rootstocks. Inoculations of 'Mondragon' with *A. mexicana* resulted in significantly lower values for RL ($P < 0.05$) compared to trees inoculated with *A. mellea* (Table 2).

3.2. Susceptibility of rootstocks inoculated with Armillaria mexicana in the field

Of the three rootstocks, 'Mondragon' showed the highest resistance to *A. mexicana* in all field evaluations (2013–2015) at 40, 59, and 66 months post-inoculation, which was displayed as the lowest values on weighted severity (WAS = 1.1, 1.3, and 1.4, respectively) and average ranks (1.5, 1.1, and 1.0, respectively) (Table 3). Symptomatic 'Mondragon' trees in the field possessed *A. mexicana*-infected lesions within the roots, and subcortical tissue above the infected area displayed a purple coloration and new roots were regenerated to replace the root losses caused by fungal infection (Fig. 1: 2a), which likely favored the survival of these infected trees. An *Armillaria* mycelial fan was observed only once in the root collar of a 'Mondragon' tree. During all evaluations, 'Criollos of La Goleta' and 'Japanese apricot' were the most susceptible and had a similar behavior ($P > 0.05$); the Armillaria root disease severity of these genotypes progressed in a similar fashion from 2014, leading to tree mortality, and all the trees of these two rootstocks had died and displayed abundant signs of *Armillaria* in 2015 at 66 months post-inoculation.

Table 3
Armillaria disease severity on three peach rootstocks evaluated 6 years after planting in an orchard naturally infested with *Armillaria mexicana* in Coatepec Harinas, State of Mexico, Mexico. August 2009 to May 2015.

Rootstocks	Disease severity[a]					Weighted average severity by rootstock (WAS)	Average ranks[b]
	0	1	2	3	4		
Disease severity 2013[c]							
'Japanese apricot'[d]	0[e]	0	2	1	4	3.3	2.7 a
'Criollos of La Goleta'[f]	2	0	3	0	2	2.0	1.8 ab
'Mondragon'[g]	2	2	3	0	0	1.1	1.5 b
Disease severity 2014[h]							
'Japanese apricot'	0	0	1	0	6	3.7	2.67 a
'Criollos of La Goleta'	0	0	1	0	6	3.7	2.46 a
'Mondragon'	2	2	2	1	0	1.3	1.1 b
Disease severity 2015[i]							
'Japanese apricot'	0	0	0	0	7	4.0	2.5 a
'Criollos of La Goleta'	0	0	0	0	7	4.0	2.5 a
'Mondragon'	2	1	3	1	0	1.4	1.0 b

[a] Individual disease severity visually assigned to five (0–4) modified classes of disease severity similar to that reported by Metaliaj et al. (2006).
[b] Average ranks with different letters are significantly different (alpha = 0.05).
[c] Disease severity observed at 40 months post-inoculation (Source: Elías-Román, 2013).
[d] *P. mume* (Japanese apricot).
[e] Number of trees in each class of disease severity.
[f] Landrace of *P. persica* (peach).
[g] *P. salicina* (Japanese plum).
[h] Disease severity observed at 59 months post-inoculation.
[i] Disease severity observed at 66 months post-inoculation.

4. Discussion

Variation in Armillaria disease incidence and severity among the tested rootstocks could be attributed to differences in virulence of the *Armillaria* species used as inoculum, natural tolerance or resistance of *Prunus* genotypes used as hosts, and/or environmental interactions. In this study, 'Mondragon' rootstock exhibited high resistance to *A. mexicana*, having the lowest infected roots incidence, and no infection by *A. mellea* was observed on this rootstock under greenhouse conditions. Similar studies have reported that hybrid plums, such as 'Marianna 2624' (*P. cerasifera.* × *P. munsoniana*), 'Ishtara'

Table 2
Effects of inoculation of three rootstocks for peach (*Prunus persica*) with two *Armillaria* species and the *Armillaria* sp.-rootstock combinations on plant growth variables at 22 months post-inoculation in greenhouse conditions (Texcoco, State of Mexico, Mexico).

Armillaria species inoculated/rootstock		n[a]	Root dry weight (RDW) (g)	Root volume (RV) (mL)	Root length (RL) (cm)	Plant dry weight (PDW) (g)
A. mexicana		21	86.0 a[b]	160.9 a	398.5 a	237.8 a
A. mellea		21	74.6 a	131.6 a	438.9 a	224.1 a
Control		12	79.8 a	131.2 a	426.3 a	249.7 a
'Nemaguard'[c]		18	79.9 b	150.4 a	389.0 b	221.6 b
'Criollos of La Goleta'[d]		18	112.2 a	187.8 a	530.4 a	333.05 a
'Mondragon'[e]		18	48.2 c	85.5 c	344.3 b	157.0 c
A. mexicana	'Nemaguard'	7	60.8 bc	123.6 cde	384.1 b	166.0 cd
	'Criollos of La Goleta'	7	159.9 a	269.8 a	622.6 a	454.4 a
	'Mondragon'	7	37.4 c	89.3 de	188.8 c	93.0 d
A. mellea	'Nemaguard'	7	87.2 b	171.4 b	397.0 b	257.4 b
	'Criollos of La Goleta'	7	87.7 b	149.8 bc	521.8 ab	264.5 b
	'Mondragon'	7	48.8 bc	73.5 e	397.7 b	150.6 cd
Control (non-inoculate)	'Nemaguard'	4	91.7 b	156.2 bc	386.0 b	241.4 bc
	'Criollos of La Goleta'	4	89.1 b	143.7 bcd	446.7 ab	280.2 b
	'Mondragon'	4	58.4 bc	93.7 cde	446.2 ab	227.4 bc

[a] Replications.
[b] In each column, means followed by different letters are significantly different, based on LS-means ($P \leq 0.05$).
[c] *Prunus persica* × *P. davidiana* (Chinese wild peach).
[d] Landrace of *P. persica* (peach).
[e] *P. salicina* (Japanese plum).

[(*P. cerasifera* × *P. salicina*) × (*P. cerasifera* × *P. persica*)], and 'Myran' [(*P. cerasifera* × *P. salicina*) × *P. persica*] showed some resistance to *A. mellea* (Cummins, 1991; Raabe, 2008); however, these rootstocks can display susceptibility when transplanted in sites infested by *D. tabescens* (Beckman and Pusey, 2001; Beckman and Lang, 2003). The greenhouse test also showed that *A. mexicana* was more virulent than *A. mellea* across all of the tested rootstocks. Although *A. mexicana* is a recently described pathogen, it is known to occur in important peach-producing regions, such as Michoacan (Rivas-Valencia et al., 2017) and State of Mexico (Elías-Román et al., 2013), but the full distribution and host range of *A. mexicana* remain largely unstudied. This research further confirms that *A. mexicana* is primary root pathogen that causes extensive damage to peach trees in Central Mexico, perhaps due to its greater distribution (Elías-Román et al., 2013) and higher virulence with respect to *A. mellea*. However, in other geographic regions, *A. mellea* has been reported as a common and aggressive root pathogen in natural ecosystems and planted crops, such as peach, grape (*Vitis* spp.), blueberry (*Vaccinium corymbosum* L.), etc. (Raabe, 1962; Guillaumin et al., 1991; Sicoli et al., 2002; Baumgartner and Rizzo, 2006; Prodorutti et al., 2009; Baumgartner et al., 2010). For this reason, it is necessary to consider the virulence and distribution of *A. mexicana* in the development of more efficient management strategies that include *Armillaria* resistant/tolerant rootstocks. Previous reports based on sites naturally infested with *D. tabescens* have attributed variation in mortality to differences in resistance among *Prunus* rootstocks (Beckman et al., 1998; Beckman and Pusey, 2001). Furthermore, hybrids of native North American plum trees [*P. angustifolia* (Marsh) and *P. umbellata*], such as 'Sharpe' rootstock, have been shown as an important source of resistance to Armillaria root disease (Beckman et al., 2008), and another *Prunus* hybrid, 'MP-29', was identified for use in *Armillaria*-infested orchards (Beckman et al., 2012). Likewise, Guillaumin et al. (1991) documented tolerance to *A. mellea* among *Prunus* rootstocks, including *P. domestica*, *P. insititia*, *P. cerasifera* ('Myrobalan'), and hybrids of these species. Subsequently, Baumgartner et al. (2018) suggested that this group of plum trees exhibits resistance, instead of tolerance, to *Armillaria*; rootstocks 'Krymsk 1', 'Krymsk 86', and 'Marianna 2624', all derived from *P. cerasifera*, have resistance to *A. mellea* and *D. tabescens*. The Japanese plum (*P. salicina*) was found as moderately resistant to *A. mellea* (Raabe, 2008).

Similar to the aforementioned reports, which indicate that several species of plum exhibit resistance to *Armillaria* spp., our study showed that 'Mondragon' (*P. salicina*; Japanese plum), propagated by stem cuttings, registered the lowest incidence of infected and dead roots (IRI, DRI) by *A. mexicana* and showed no evidence of infection when it was inoculated with *A. mellea* in greenhouse conditions. The behavior of 'Mondragon' in the greenhouse was consistent with its behavior in the field, where it also showed the lowest disease severity. These results reflect inherent resistance/tolerance attributes of this *P. salicina* genotype. The selection of both general strategies (i.e., resistance and tolerance) has been suggested previously to enhance the capacity of *Pseudotsuga menziesii* (Douglas-fir) to cope with the attack by *A. ostoyae* (= *A. solidipes*) (Cruickshank and Jaquish, 2014). In plum trees (*P. cerasifera*, *P. domestica*, *P. insititia*, and hybrids of these species), activation of defense reactions in the sapwood and bark appear to limit colonization by *A. mellea* and are considered as resistance attributes (Guillaumin et al., 1991; Baumgartner et al., 2018). However, the presence of these attributes could perhaps result in reduced growth of healthy and/or diseased plants. For example, *P. menziesii* plants that exhibited the capacity to limit *A. ostoyae* colonization within only small lesions in the affected area, exhibited less radial growth (Cruickshank and Jaquish, 2014). Also, peach plants grafted on plum rootstocks 'Sharpe' and 'MP-29', which are resistant to *D. tabescens*, showed lower stem diameter than plants grafted on susceptible rootstocks, such as 'Guardian' (*P. persica*) propagated by seeds (Beckman et al., 2012). Similarly, the resistant trees of 'Mondragon' had lower values for dry weight of the whole plant and roots compared to most susceptible peach rootstocks, which were propagated by seed.

In relation to Japanese apricot (*P. mume*) rootstock, vegetative compatibility with peach and selections resistant to the plant parasitic nematodes *Meloidogyne javanica* and *M. incognita* has been previously reported by Mayer et al. (2003, 2005). Raabe (2008) also mentioned that *P. mume* is moderately resistant to *A. mellea*; however, this study is the first to document the high susceptibility of *P. mume* to *A. mexicana*.

Tolerance to infection by *A. mellea* was reported in rootstock 'GF677' (*P. persica* × *P. amygdalus*) based on the balance between speed of invasion by the fungus and the speed the host to replace the roots affected by disease (Guillaumin et al., 1991). This response was observed with 'Criollos of La Goleta' inoculated with *A. mexicana* under greenhouse conditions, where this rootstock produced higher root volume (RV), root dry weight (RDW), and plant dry weight plant (PDW) in comparison to non-inoculated plants (Table 2). However, *A. mexicana* mycelium was observed around the root collar of 'Criollos of La Goleta' inoculated under greenhouse conditions, and all infected plants had died by the end of the evaluation period under field conditions.

Another resistance/tolerance mechanism that could have affected 'Mondragon' survival in field was observed as a vertically inverted lesion resulting from *Armillaria* infection at the root collar. A similar resistance/tolerance mechanism has been observed in *Armillaria*-infected plants of *P. menziesii* that exhibited high survival rate under greenhouse and field conditions (Cruickshank and Jaquish, 2014). In those plants, root collar girdling was limited because lesion spread was restricted horizontally; whereas, trees that formed horizontal lesions resulted in trunk girdling and subsequent tree mortality.

In conclusion, this study contributes to management strategies for Armillaria root disease of peach in Mexico by providing considerations for deployment of resistant rootstock, such as 'Mondragon', that could provide better performance within a scheme of integrated management that includes other disease management strategies, such as excavating root collars above ground level (Schnabel et al., 2012) and inoculum reduction measures (Cox et al., 2005b). Additionally, it is critical to determine which *Armillaria* spp. are present in the production areas, due to virulence variation within genus *Armillaria*; different *Armillaria* species represent distinct threats for different hosts and geographic areas. Because *A. mexicana* appears as the primary pathogen of peach orchards in central Mexico, it is essential to consider this pathogen for the disease management strategies. Moreover, all management strategies for Armillaria root disease should be validated according to the specific features that characterize the production areas, such as climate, soil types, technology, and other environmental factors.

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