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Research Article

Mycorrhizal fungi in *Annona muricata* L. rhizosphere in two agricultural production systems in Nayarit, Mexico

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Abstract

Mycorrhizal symbiosis is the association between the roots of several plant species and soil fungi of the *Glomeromycotina subphylum*. This symbiosis plays a crucial role in plant growth, development, and defense; therefore, understanding the abundance and diversity of arbuscular mycorrhizal fungi (AMF) species associated with plants of economic importance is of utmost concern. The objective of this study was to compare the abundance and composition of AMF communities in two *Annona muricata* cultivation sites, and in propagated soil, under different agricultural practices. The first cultivation site is under technified management with periodic fertilization and irrigation (TS), while the second cultivation site is under agroecological management without fertilization, irrigation, and with the presence of livestock (non-technified site; NTS). The extracted spores from the collected samples and the trap cultures were taxonomically identified based on their morphology. 13 species associated with *A. muricata* belonging to seven genera were identified in soil samples. The most abundant species were *Funneliformis geosporum*, *Acaulospora kentinensis* and *Rhizophagus intraradices* with a relative abundance of 45.9, 19.0 and 15.8%, respectively. In the propagation substrates, only 69% of the AMF species found in the field were identified. 11 species of AMF were found in the site with non-technified agronomic management, while only five species were found in the technified site.

Keywords: AMF diversity; AMF propagation; Mycorrhiza; soursop

Introduction

Arbuscular mycorrhizal fungi (AMF), *Glomeromycotina subphylum*, are obligate symbionts of a wide variety of host plants (Tedersoo *et al*., 2018). AMFs are the only fungal group capable of forming spores larger

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than 500 μm in diameter with high storage of lipids, some carbohydrates, and have thick walls which contain chitin and, in some cases, β (1-3) glucan and mannans (Smith and Read, 2008; Hai-Ru *et al*., 2011; Balestrini and Bonfante, 2014). These thick and complex walls allow glomeromycete spores to withstand heat, low soil water content, and a wider range of soil pH than other fungal groups (Giovannetti, 2000). In several genera and species, latency has been reported, some studies reveal that diverse plant communities can support AMF viability in soil for extended periods (Dietrich *et al*., 2020). Besides, AMF spores form several germination hyphae at the same time -multiple germination- and can retract the cytoplasmic content of a germination hypha towards the spore, returning to dormancy and subsequently producing a new germination hypha (Giovannetti, 2000). These features make spores the most significant structure of propagation, dispersion, and resistance of glomeromycetes and, therefore, an essential element for their management. For decades it has been recognized that mycorrhizal symbiosis plays an essential role in plant growth and bio-protection, besides improving soil quality (Dalpé and Monreal, 2004). Mycorrhization generally promotes the growth of many plant species; numerous studies have reported the influence of AMFs on plant development and growth (Smith and Read, 2008), from which most commercial interest crops, such as corn, pepper, beans, agave, cotton, guava, orange, sour orange, apple, among others (Dalpé and Monreal, 2004; Grzyb *et al*., 2015; Reyes-Tena *et al*., 2016; Ortas *et al*., 2016; Ying *et al*., 2017; Trinidad-Cruz *et al*., 2017a; Gao *et al*., 2020; Quiñones-Aguilar *et al*., 2020). Therefore, AMFs have been proposed over the years as growth promoters in plants (Qiang-Sheng *et al*., 2019).

Although the same species of AMF may be associated with different hosts, some AMF may obtain greater or less benefit from one host than another (De La Rosa-Mera *et al*., 2012). From this assumption arise the interest and importance of obtaining and selecting native AMF strains and consortia for application in plant species or under specific climatic conditions and obtaining the specific benefits of this symbiosis (Rodríguez *et al*., 2004; Trejo *et al*., 2011). Moreover, some agronomic practices, such as the establishment of monocultures, tillage practices, and the application of fertilizers, pesticides, and fungicides can negatively affect the diversity of AMF. Regarding growing a single crop, the dominance of a plant species produces specific root exudates, generating certain selectivity of AMF species. Besides, tillage practices can decrease the AMF spore density and alter the interconnection network of hyphae in the soil; in the same way, the application of fertilizers negatively affects the development of AMF as well as its abundance and diversity in the agroecosystems (Agnihotri *et al*., 2017). Trap cultures have been used to know AMF abundance and diversity of several ecosystems and field sites; however, AMF abundance and diversity may differ from the sampling sites due to the recovery of additional AMF species that were not identified at the sampling time (Leal *et al*., 2009). Additional AMF species in the traps is a common phenomenon, given the different environmental conditions in trap cultures in comparison to the fields, some of the AMF rarely sporulating in the field soil, might start forming spores in the trap cultures (Leal *et al*., 2009; Songachan and Kayang, 2014); nevertheless, also an opposite effect may occur, the initial AMF species that are not tolerant to the trap conditions will not be propagated or will be propagated in a smaller proportion (Trejo-Aguilar *et al*., 2013).

However, there are still many cultivated plants in which the native AMF species of their rhizospheres and the effects that can affect their growth are unknown. One of them is *Annona muricata*, known as soursop. This fruit tree is native to tropical America and is currently cultivated in tropical regions of America, Asia, and Africa; this species is appreciated for its edible fruit (Cordero and Boshier, 2003; Hernández-Fuentes *et al*., 2017). In Mexico, it is cultivated in tropical areas, from Sinaloa to Chiapas, in Morelos and from Veracruz to Yucatán (Reyes-Montero *et al*., 2018). Although there are no reports of the mycorrhizal status of *A. muricata*, the presence of arbuscular mycorrhiza in various genera and species of Annonaceae, including *A. cherimola* (Wang and Qiu, 2006), suggest the existence of this association in *A. muricata* and its possible influence on plant growth. However, to address this latter aspect, it is necessary to obtain biological material from this fungi group. The aim of this study is to compare the abundance and composition of AMF communities present in the soursop rhizosphere, as well as in the propagated soil, from two cultivation sites with different agronomic management. The first cultivation site is under conventional or technified management (TS) with periodic

fertilization and irrigation, while the second cultivation site is under agroecological management (NTS) without fertilization, irrigation, and with the presence of livestock. We hypothesize that AMF abundance in the rhizosphere of *A. muricata* will be higher in unmanaged cultivations sites compared to high managed agricultural sites.

Materials and Methods

Study sites and sampling

The study sites were in the municipality of Compostela, in the state of Nayarit, Mexico. Sampling was carried out in two cultivation areas of *Annona muricata* under different intensities of agronomic management. The first site called technified site (TS) is located at the geographical coordinates of 21° 07′ 35.2′′ N and 105° 12′ 12.5′′ W, at an altitude of 148 m above sea level, has an area of 10 ha and is located on a 15% slope. It is characterized by extensive management, with periodic fertilization and irrigation. The second site, called non-technified site (NTS), is located at the geographic coordinates of 21° 12′ 48′′ N and 105° 03′ 10′′ W, at an altitude of 188 m above sea level, has an area of 5 ha and is located on a 40% slope; agro-ecological management, without fertilization or irrigation and with the presence of livestock.

Samples of *A. muricata* rhizospheric soil (adjacent to tree roots) were obtained in May 2018 from both study sites. The trees were randomly sampled: four trees from the technified site (TS) and five from non-technified site (NTS). For each specimen, four subsamples were taken from 0 to 15 cm deep. The subsamples were mixed, forming a final sample of 5 kg per tree. The samples were dried and stored at room temperature, protected from sunlight for later analysis.

Physical and chemical analysis of the soursop rhizosphere and the substrate for AMF propagation

The determination of P, N, K and Ca in soil was carried out through extracts obtained from wet digestion, which was made by adding 4 mL of H_2SO_4 and $HClO_4$ (2: 1, respectively) to 500 mg of rhizospheric soil that was left undisturbed for 24 h, then H_2O_2 (30%) was added and digested until reaching 200 °C, once the organic matter had disintegrated, the extracts were washed. The determination of N was made by the Kjeldahl method (Kirk, 1950). P was determined by the ammonium molybdate colorimetric method (Chapman and Pratt, 1979). The pH was quantified through a potentiometer from a mixture of water and soil (ratio 2:1, v/v).

A sample of the trap culture substrate propagated soil was analyzed to know its P, N, K and Ca contents, in the Agricultural Analysis Laboratory of the Fertilab® company in Celaya Guanajuato (fertilab.com.mx) according to the procedures of the Official Mexican Standard NOM-021-RECNAT-2000 (2002).

Isolation, taxonomic identification, and determination of AMF diversity

AMF spores were extracted from 50 g of dry soil of each sample through wet sieving and decantation (Gerdemann and Nicolson, 1963), then they were centrifuged in 50% sucrose (Brundrett *et al*., 1996). The spores were selected, extracted and placed on a slide, a drop of polyvinyl alcohol in lactoglycerol (PVLG) mixed with Melzer's reagent in a 1:1 ratio was added and spores were observed using an optical microscope (Leica, DM750, Wetzlar, Germany) to know and describe the components of the cell wall (Tapia-Goné *et al*., 2008; Trinidad-Cruz *et al*., 2017a). For taxonomic identification of AMF species, the morphological characteristics observed were contrasted against those described in magazines and in some specialized web pages such as Arbuscular Mycorrhizal Fungi Phylogeny (http://www.arbuscular-mycorrhiza.net/amphylo_home.html), International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (http://wwwinvam.wvu.edu) and Professor Janusz Blaszkowski, taxonomist of Glomeromycota (http://www.zor.zut.edu.pl/Glomeromycota). The classification proposed by Schüβler *et al*. (2001) and

Redecker *et al*. (2013) was also used. The absolute abundance was obtained by counting the number of AMF spores per specie, whereas relative abundance was estimated by dividing the number of AMF spores of each species by the total abundance of all AMF species identified. Simpson and Shannon-Weaver diversity indices were calculated, and the ecological diversity of both study sites was compared by a T-Hutchenson test ($p \leq 0.05$).

Photographs of some species were taken with a digital camera integrated into the microscope (Leica, ICC50E, Wetzlar, Germany) and the Leica Application Suite V 3.3.0 software (Leica Microsystems, 2016).

Colonization rates and spore density of arbuscular mycorrhizal fungi (AMF)

Spores were extracted from 100 g of dry soil through sieving and decantation (Gerdemann and Nicolson, 1963), and the samples were centrifuged in a 50% sucrose gradient. Later, spores were observed and counted under a stereoscopic microscope. Roots were placed in plastic cassettes and subsequently embedded in KOH (10%) overnight, then KOH was placed again (10%) and were autoclaved (5 min, 121 °C, 1.0546 kg cm^{-2}); subsequently, the roots were washed with water and placed in H_2O_2 (10%) for 5 min (100 °C), roots were rewashed with water and placed in HCl (1N, room temperature, 10 min) after that, roots were stained with trypan blue (0.02%, 95 °C, 10 min); root sections were mounted on slides. We determined the percentage of colonization according to the method described by McGonigle *et al*. (1990) and it was determined by different structures such as arbuscules (A), coils (C), and vesicles (V); hyphal colonization was expressed as total colonization. The colonization percentage, and the number of spores from both study sites, were compared using a T-Student test ($p \leq 0.05$).

AMF spore propagation

Rhizospheric soil of each sample (50 g) was placed in trap cultures with *Sorghum tricolor* (L.) Moench (sorghum), *Trifolium* sp. (clover) and soursop seedlings (*A. muricata*) as trap plants. The trap plants and the inoculum were placed in pots with 5 kg of a sterilized substrate (121 °C, 1.05 kg cm^{-2}, 6 h) of a mixture of sand, soil and perlite in proportions 2:1:1 (v:v:v). The native AMF inoculum was placed between two layers of the substrate. The pots were watered to field capacity as needed and were kept in greenhouse conditions (minimum temperatures of 10-12 °C and maximum of 26 to 30 °C) for seven months to favour the establishment and sporulation of the AMF present in the base inoculum. Subsequently, the extraction and taxonomic identification of the spores from the trap cultures were carried out.

Statistical analysis

The P, N, K and Ca content in both sampled soils (TS and NTS) were compared using the Kolmogorov-Smirnov statistical test ($p \leq 0.05$), the determination of differences between treatments was conducted by comparing the medians with the Statgraphics statistical program Centurion XV version (Statpoint, 2005).

Results

AMF spore propagation

The pH levels at both sampling sites were statistically similar, with a pH of 4.8 at the technified site (TS) and 5.3 at the non-technified site (NTS), as shown in Table 1. Also, the N content in TS and NTS were statistically similar (3850 mg kg^{-1} on TS and 3920 mg kg^{-1} on NTS; Table 1). Otherwise, TS showed a higher total P content (1084 mg kg^{-1}) than NTS (558 mg Kg^{-1}; Kolmogorov-Smirnov; $p \leq 0.05$; Table 1). In addition, NTS showed a higher K and Ca content than TS (Kolmogorov-Smirnov; $p \leq 0.05$; Table 1).

Table 1. Physical and chemical characteristics of the *Annona muricata* rhizosphere soil from two commercial cultivation sites in the municipality of Compostela, Nayarit and the substrate used in the propagation of AMF

Sample	pH	P	N	K	Ca
		(mg kg^{-1})			
TS	4.8±0.1a	1084±46a	3850±40 6a	646±192b	12130±1102b
NTS	5.3±0.2a	558±41b	3920±182a	9715±806a	18526±1650a
Trap culture substrate	6.7	32.2	11	140	376

P: phosphorus; N: nitrogen; K: potassium; Ca: calcium. Different letters indicate statistical differences according to the Kolmogorov-Smirnov test ($p \leq 0.05$) between cultivation sites with technification (TS) and without technification (NTS), ± standard error.

Isolation and taxonomic identification of AMF

In the nine samples from rhizospheric soil of *A. muricata*, the total spore AMF richness was 13 species belonging to seven genera, three families and two orders: *Acaulospora*, *Claroideoglomus*, *Funneliformis*, *Rhizophagus*, *Sclerocystis*, *Scutellospora* and *Septoglomus* (Table 2; Figure 1). Five species were found at the TS site, while 11 were found at the NTS site. The most abundant species in TS site were *Funneliformis geosporum*, *Acaulospora kentinensis* and *Acaulospora spinosa* with a relative abundance of 60.0, 17.14 and 14.28%, respectively (Table 2), whereas in NTS site the most abundant species were *F. geosporum*, *A. kentinensis* and *Rhizophagus intraradices* with a relative abundance of 43.57, 19.26 and 18.34%, respectively (Table 2).

Table 2. Absolute and relative abundance of arbuscular mycorrhizal fungal species (AMF) present in the *Annona muricata* rhizosphere at two commercially cultivated sites (Technified Site: TS and Non-Technified Site: NTS) in the soursop producing zone in Compostela, Nayarit.

Order	Family	Species	Site	Abundance*	
				A	R
Diversisporales	Acaulosporaceae	*Acaulospora excavata* Ingleby & C. Walker	TS	0	0
			NTS	1	0.45
		Acaulospora kentinensis Kaonongbua, Morton & Bever	TS	6	17.14
			NTS	42	19.26
		Acaulospora mellea Spain & N.C. Schenck	TS	0	0
			NTS	1	0.45
		Acaulospora morrowiae Spain & N.C. Schenck	TS	0	0
			NTS	4	1.83
		Acaulospora spinosa C. Walker & Trappe	TS	5	14.28
			NTS	0	0
	Gigasporaceae	*Scutellospora dipurpurescens* J.B. Morton & Koske	TS	2	5.71
			NTS	13	5.96
Glomerales	Claroideoglomeraceae	*Claroideoglomus claroideum* C. Walker & A. Schüßler	TS	0	0
			NTS	5	2.29
		Claroideoglomus etunicatum C. Walker & A. Schüßler	TS	0	0
			NTS	5	2.29
	Glomeraceae	*Funneliformis geosporum* C. Walker & A. Schüßler	TS	21	60
			NTS	95	43.57
		Rhizophagus fasciculatum C. Walker & A. Schüßler	TS	0	0
			NTS	3	1.37
		Rhizophagus intraradices C. Walker & A. Schüßler	TS	0	0
			NTS	40	18.34
			TS	1	2.85

		Sclerocystis sinuosa Gerd. & B.K. Bakshi	NTS	0	0
		Septoglomus constrictum Sieverd., G.A. Silva & Oehl	TS	0	0
			NTS	9	4.12

*A= absolute; R= relative. The species nomenclature follows to Schüβler and Walker (2010) and Redecker *et al.* (2013).

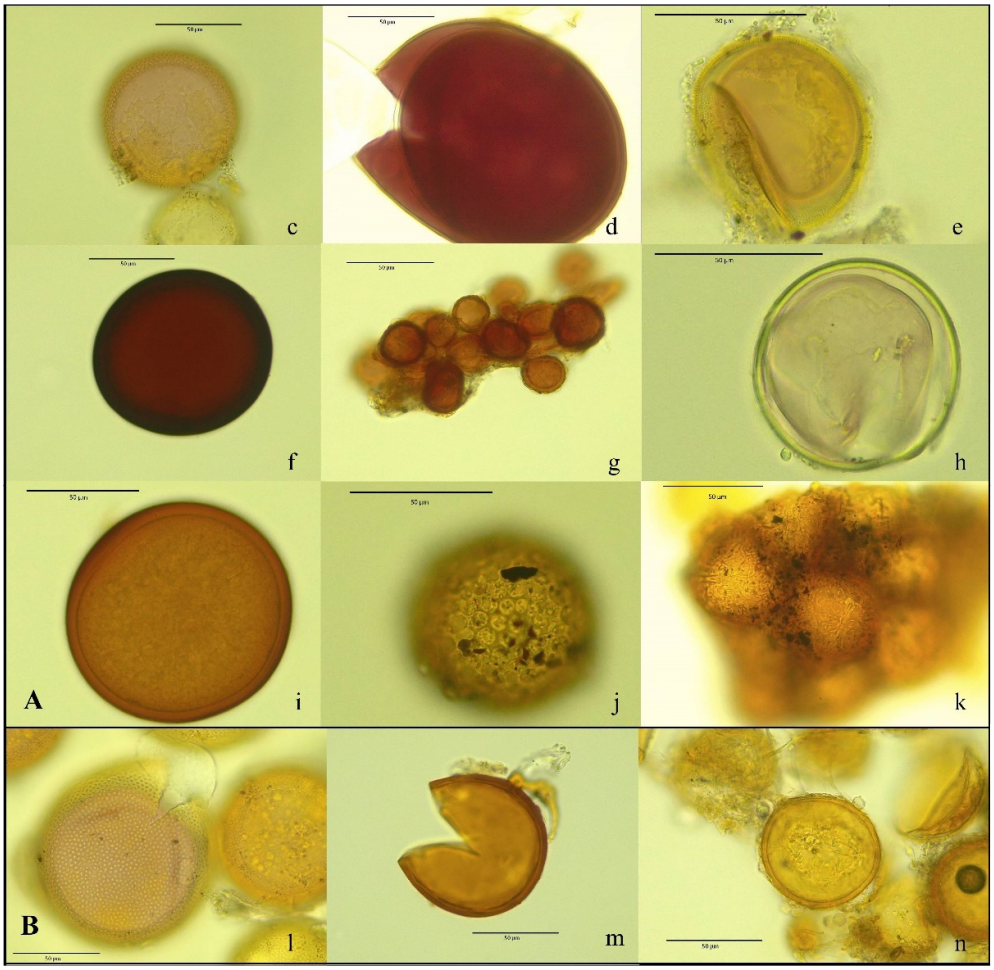

Figure 1. Species of arbuscular mycorrhizal fungi (AMF) identified in the *Annona muricata* rhizosphere at two commercial cultivation sites in Compostela, Nayarit (**A**: c-k) and in the trap cultures (**B**: l-n). c. *Acaulospora kentinensis* (AMF collected); d. *Scutellospora dipurpurescens*; e. *Acaulospora spinosa*; f. *Septoglomus constrictum*; g. *Rhizophagus fasciculatum*; h. *Acaulospora morrowiae*; i. *Claroideoglomus etunicatum*; j. *Acaulospora excavata*; K. *Sclerocystis sinuosa*; l. *Acaulospora kentinensis* (AMF propagated); m. *Funneliformis geosporum*; n. *Rhizophagus intraradices*; j. *Claroideoglomus claroideum*. 50 µm bar

F. geosporum, *A. kentinensis* and *S. dipurpurescens* were found in the rhizospheres of *A. muricata* from both sites, under different agronomic management, while *A. excavata*, *A. mellea*, *A. morrowiae*, *C. claroideum*, *C. etunicatum*, *R. fasciculatum*, *R. intraradices* and *S. constrictum* were only found at the NTS site (Table 2).

The present study shows that the NTS site has a greater abundance and species richness (Table 3); likewise, the Shannon-Weaver index shows a higher diversity in the NTS site compared to the TS site (2.3732 and 1.6619, respectively; $p<0.05$, T-Hutchenson). Regarding the Simpson index (dominance index), the HT field shows a greater dominance than the HNT field (0.4138 and 0.2675, respectively; Table 3).

Table 3. Species richness, abundance, diversity indices, and expected species richness of AMF spores at two production sites of *Annona muricata* in Compostela Nayarit

Diversity parameters	Sample sites		Significance (p-value)
	TS	NTS	
Species richness (S)	5	11	0.0040
Simpson index (D)	0.4138	0.2675	0.0821
Shannon-Weaver index (H´)	1.6619	2.3732	0.0043

Colonization rates and spore density of arbuscular mycorrhizal fungi (AMF)

In the TS field, AMF spore density was lower (277 ± 24 spores 100 g of dry soil; Table 4) than in the NTS field (618 ± 36 spores 100 g of dry soil; Table 4). Mycorrhizal colonization percentages were also different in both sites; in the TS field, a total colonization of 32 ± 2% was observed of which 5.5 ± 3% comprises colonization by "coils", 2.44 ± 1% by vesicles and 3.0 ± 1% by arbuscules; in the NTS field, a total colonization percentage of 58 ± 6% was determined, 22 ± 7% comprises colonization by "coils", 1.2 ± 0.3% by vesicles and 26 ± 8% by arbuscules (Figure 2, Table 4).

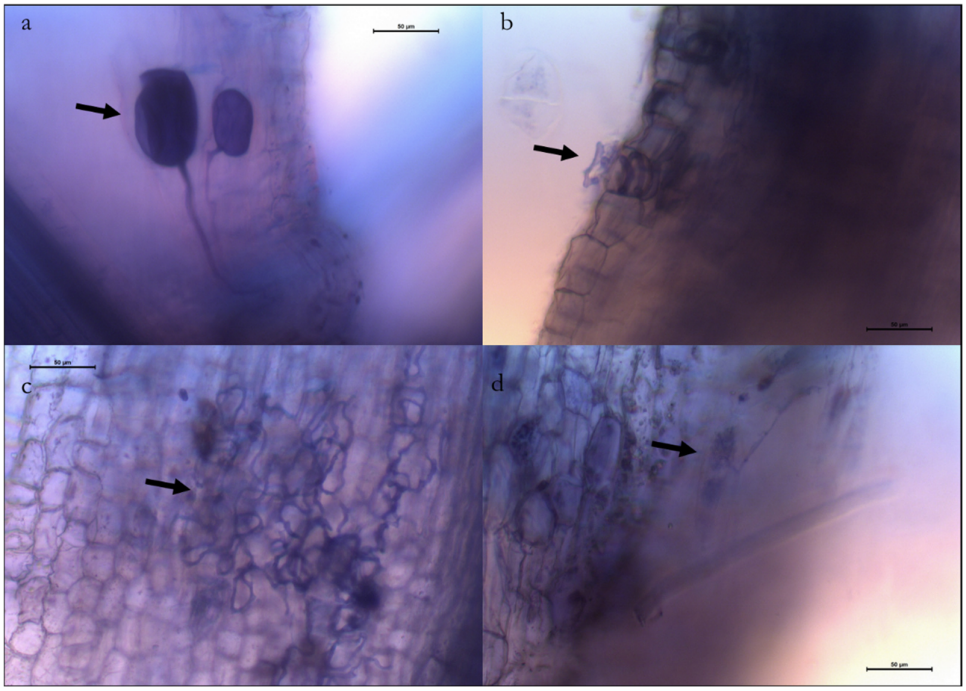

Figure 2. AMF structures in soursop roots (*Annona muricata*) at two production sites in Compostela Nayarit. Arrows indicate vesicles (a), hyphae (b and c) and arbuscules (d) of AMF in roots of *A. muricata* clarified and stained with trypan blue 0.02%. Bars 50 µm. The arrows show the structures indicated in the paragraph

Table 4. Determination of the spore density and percentage of colonization of AMF in production sites of *Annona muricata* in Compostela Nayarit

Sample sites	Mycorrhizal colonization (%)				Spore density (100 g dry soil)
	Coils	Vesicles	Arbuscules	Total (hyphal)	
TS	5.5±3a	2.4±1a	3.0±1a	32±2a	277±24a
NTS	22±7a	1.2±0.3a	26±8b	58±6b	618±36b

Different letters indicate significant differences according to the T-Student test ($p \leq 0.05$); ± standard error.

AMF species richness in trap cultures

AMF species propagated in soursop rhizosphere soil trap cultures (TS and NTS) after 7 months are shown in Table 5. As a result of AMF propagation, only 9 species of the 13 found in field samples of both sites were registered (69%). In the TS site trap cultures, a total of 8 species were identified, including the species *A. morrowiae*, *C. claroideum*, *C. etunicatum*, *R. intraradices* and *S. constrictum*, which were registered as exclusives species of the NTS sampling site (Table 5). On the contrary, in the NTS site, 9 species were propagated, of which *A. spinosa* had only been identified in the TS sampling site (Table 5).

Changes in the species composition of the AMF community were observed at both sites through propagation in trap cultures; the number of species shared between both sites increased from 3 to 8 species (Table 5).

Table 5. Distribution of AMF species present in the *Annona muricata* rhizosphere in two commercial cultivation areas in the municipality of Compostela, Nayarit (AMF collected), and AMF species present in the trap cultures (AMF propagated)

Sample site	AMF collected	AMF propagated
TS	13 species	9 species
	A. spinosa	NES
	S. sinuosa	
NTS	*A. excavata*	*S. dipurpurescens*
	A. mellea	
	A. morrowiae	
	C. etunicatum	
	C. claroideum	
	R. intraradices	
	R. fasciculatum	
	S. constrictum	
Both sites	*A. kentinensis*	*A. spinosa*
	F. geosporum	*A. morrowiae*
	S. dipurpurescens	*A. kentinensis*
		C. etunicatum
		C. claroideum
		F. geosporum
		R. intraradices
		S. constrictum

NES: Not exclusive species; TS: Techinified site; NTS: Non-technified site

The abundance of spores of AMF species in the samples from soursop rhizosphere and during propagation in trap cultures are shown in Figure 2. The most abundant species present in the trap cultures of both sites were *F. geosporum* and *R intraradices*. Regarding *F. geosporum* species, 19 spores were recorded in 50 g of dry soil at the TS site and 128 spores in 50 g of dry soil at the NTS site, while for *R. intraradices* 5 spores were recorded in 50 g of dry soil at the TS site and 117 spores in 50 g of dry soil at the NTS site (Figure 3).

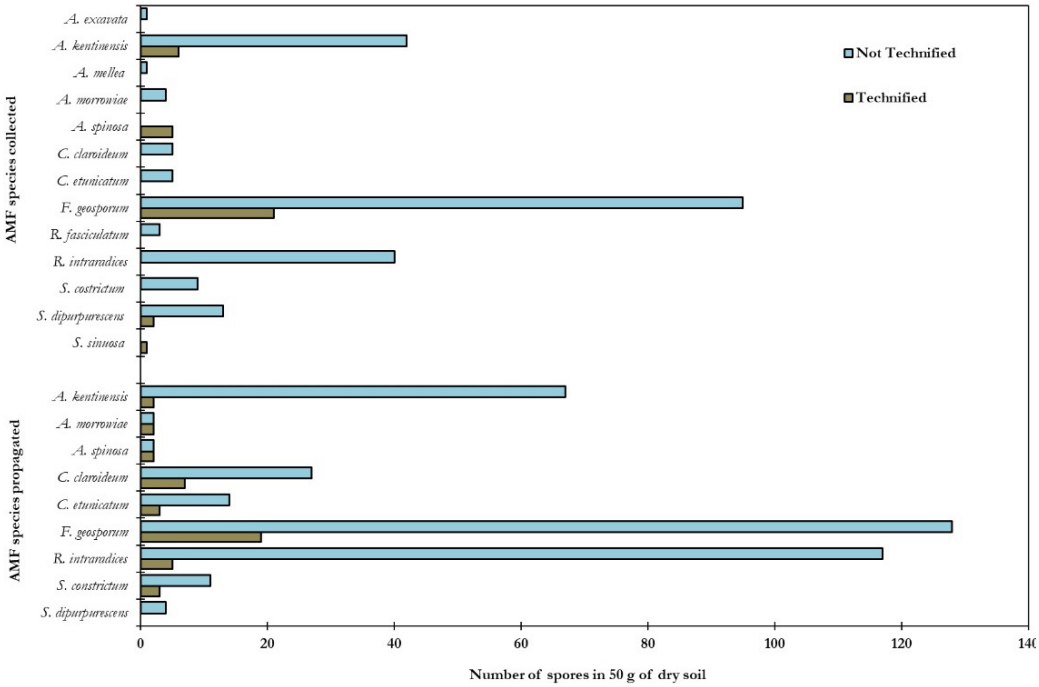

Figure 3. Absolute abundance of arbuscular mycorrhizal fungi (AMF) spores in rhizosphere soils of *Annona muricata* from two cultivation areas in Compostela, Nayarit, Mexico (AMF species collected), and in trap cultures made with the same soils (AMF species propagated)
The graph displays the relationship between the number of spores found in the soil (x-axis) and the identified species (y-axis).

Discussion

The edaphic characteristics of both sampling sites showed significant differences in parameters such as P, K and Ca; the observed difference is due to the intensity of the agronomic management that both sites receive. While the TS site receives conventional management, the NTS site receives agroecological management, without the application of chemical fertilizers, evidenced by the low concentration of P in the soil. Surprisingly, a greater content of Ca and K has been found at the NTS site, however, this result is opposed to the ones reported in a study comparing organic and conventional systems that demonstrates that nutrient inputs (N, P, K) content was 34 to 51% lower in the organic farming sites, whereas Ca and Mg content were 30 to 50% higher in the organic farming sites (Mäder *et al*., 2002). Animal-based organic fertilizer sources can remarkably impact soil chemical properties (Leskovar and Othman, 2018); hence, is presumed that the high content of Ca and K in the NTS site may be due to the presence of cow manure. Uzoma *et al*. (2011) have shown that cow manure biochar improved exchangeable cation (K, Ca, and Mg) status of the soil. Also, Amiri and Fallahi (2009) reported in an apple orchard soil, the increase of K and Ca, whereas P content kept the same, due to cow and poultry manure addition.

In our current investigation we found that spore density was higher in the NTS field as shown in Table 4. This finding is consistent with Toprak (2017), who found a higher spore density in low-input farming systems. Similarly, previous studies have indicated that the intensive agricultural methods have a detrimental effect on spore density (Elbon *et al*., 2014). Regarding the level of mycorrhizal colonization, various research findings demonstrate elevated colonization rates in organic farming systems or unconventional management fields (Knerr *et al*., 2019), aligning with the outcomes presented in our study. The higher spore density and

colonization rates observed in HNT may be attributed to agronomic management practices. Conventional agricultural practices such as crop rotation, tillage, fertilizer and pesticide application, soil aggregation, and cultivation of genetically modified plants are known to impact AMF (Agnihotri *et al.,* 2017).

The AMF species richness, absolute abundance and diversity indices were different at both sites. A higher number of species, greater absolute abundance and diversity were observed at the NTS site, under agroecological management. This result indicate that the type of agronomic management applied to crops influences the diversity, richness and abundance of AMF in the rhizosphere. Even though the influence of some fertilizers may be variable in AMF community, the high levels of some mineral nutrients in soil, like P, could be reducing the AMF richness and abundance in the TS site. Some authors such as Dai *et al.* (2014) have reported differences in the number of native AMF species in wheat crops with conventional and organic management, recording a 32.5% greater richness of AMF species in the organic site, along with increased Simpson and Shannon-Weaver diversity indices. Also, Dai *et al*. (2014) observed a higher percentage of root colonization in organic sites (23.1%) than conventional sites (9.3%). Likewise, Oehl *et al*. (2003) reported that the type of agronomic management of different agroecosystems is directly related to the decrease in AMF species richness. At the same study, Oehl *et al.* (2003) observed 20.5-17.0 AMF species in extensive grassland sites, while in crop rotation and monocropping field sites 15.5-11.2 and 8.0-6.0, respectively, AMF species were found; as expected, the diversity of AMF was higher in the crop rotation sites. In Mexico, similar results related to agronomic management were reported by Carballar-Hernández *et al.* (2013) for soils cultivated with *Agave potatorum*. These results are attributed to the low levels of mineral nutrients present in organic or agroecological crops since it has been reported that the use of mineral fertilizers and synthetic pesticides can negatively influence the structure of the native AMF community (Jansa *et al*., 2006; Carballar-Hernández *et al*., 2013; Carballar-Hernández *et al*., 2017). However, other factors must be included in studies related to the determination of richness, diversity and composition of native AMF species present in agroecosystems, factors such as tillage, crop rotation, cultivation of non-myco-trophic species, history of the site, soil aggregation and application of fungicides (Hijri *et al*., 2006; Agnihotri *et al*., 2017; Carballar-Hernández *et al*., 2017).

In this work, a richness of 13 species associated with the *A. muricata* rhizosphere was recorded at two sites with different agronomic management in soursop producing regions of Compostela's municipality, Nayarit. The present study is the first report about the richness and abundance of AMF species associated with the *A. muricata* rhizosphere in Mexico and the rest of the world. Also, this paper is one of the first reports, together with Lauriano-Barajas and Vega-Frutis (2018), about AMF richness in Compostela, Nayarit. Lauriano-Barajas and Vega-Frutis (2018) reported 9 AMF species (*C. etunicatum*, *S. constrictum*, *Claroideoglomus* sp., *Acaulospora* sp. and 5 unidentified species) for a tropical montane cloud forest located in the municipality of Compostela, Nayarit; two of these species (*C. etunicatum* and *S. constrictum*) were also identified in the rhizospheric soils of *A. muricata* and propagated from the same soils in this study. There are few reports related to AMF species of the rhizospheres of species of the *Annona* genus. *Glomus aggregatum*, *Claroideoglomus claroideum* (cited as *Glomus claroideum*) and *Acaulospora spinosa* are the registered AMF species of the *Annona squamosa* rhizosphere so far (Ragupathy and Mahadevan, 1993; Bhale *et al*., 2011).

The establishment of trap cultures is one of the best strategies to isolate and spread AMFs from different plant species or ecosystems of interest; it has advantages and limitations. Among the limitations, trap cultures do not always promote the sporulation of all AMF species recorded at a sampling site because sporulation cycles of some AMF can be altered by the host plants chosen for their propagation. Moreover, AMF propagules of the plant or site of interest are not present or are insufficient for further propagation. In contrast and from a favorable point of view, trap cultures allow sporulation of AMF species not sporulating at the time of sampling (Leal *et al*., 2009). In this study, the species richness of AMF in the trap cultures decreased after seven months. In the trap cultures, only 69% of the AMF species found in the *A. muricata* rhizospheres were conserved in the field, under either culture condition. The loss of AMF species due to one or subsequent growing cycles in trap

cultures is a phenomenon that has been reported by various authors. Bever *et al*. (1996) reported a lower richness of AMF species in trap cultures established with sorghum than the richness found in isolated field samples of *Anthoxanthum*, *Panicum* and *Plantago* and successive propagation crops with the same species. Propagation of native AMF inocula in trap cultures systems presents several difficulties. Natural environmental conditions of source sites cannot be recreated in a greenhouse, so species that tolerate the new growth conditions proliferate more (Trejo-Aguilar *et al*., 2013). In contrast, species that are not tolerant to new growth conditions will be propagated in a smaller proportion or even not be propagated (Trejo-Aguilar *et al*., 2013). Other factors may also influence the number of AMF species propagated, for example, selected host plant species, propagation substrate mix, and time and management of trap cultures (Yao *et al*., 2010). Regarding the role of interactions between AMF, little is known about the competition (Pearson *et al*., 1994; Cano and Bago, 2005) and codependency between species, ecological behaviors that can influence propagation.

Combinations of these factors can create favorable selection pressures for some AMF species and unfavorable for others. In this work it is determined that the proportion of *F. geosporum*, one of the most abundant species in the native rhizospheric soils, was similar between the rhizospheric soil sample and the trap cultures; whereas, *C. claroideum* and *C. etunicatum* increased their proportion in the trap cultures. This last species has been reported to be able to adapt to conditions very different from those of where it was isolated, in addition to being tolerant to changes of the host plant (Manoharan *et al*., 2008; Trejo-Aguilar *et al*., 2013) while *C. claroideum* and *F. geosporum* show to be species with broad adaptation capacities to different cultivation and management practices (Carballar-Hernández *et al*., 2017; Oehl *et al*., 2017). The dominant species in the sampling sites of *A. muricata* rhizosphere and in the trap cultures are *F. geosporum* and *R. intraradices* (Figure 2). These species, like *C. claroideum* and *C. etunicatum*, are members of the Glomerales order. Several authors have pointed out the dominance of AMF species belonging to this order during propagation (Kennedy *et al*., 2002; Trejo-Aguilar *et al*., 2013; Trinidad-Cruz *et al*., 2017b), for considering as generalists and highly competitive AMF species (Córdoba *et al*., 2001).

Conclusions

The specific richness of AMF present in the *A. muricata* rhizosphere in the cultivation areas of Nayarit's state was 13 species. The most significant number of AMF species (11 species) was recovered from the agroecological management site, while sites under agronomic management had only 5 species. Species composition and absolute spore abundances changed after seven months of propagation, remaining only 69% of the species found at both sites. The species of the Glomerales order were the dominant ones during the propagation. Although the results of this study show the viability of propagating native AMFs from *A. muricata* rhizospheric soils, it is necessary to carry out several successive subcultures to create a stable AMF consortium. Further work is needed to explore the ability of this native AMF species to promote arbuscular mycorrhiza structures in *A. muricata*, and therefore, provide the benefits derived from this association.

Authors' Contributions

Conceptualization: AMGL, GRE, GAS and EEQA; Data curation: AMGL and LVHC; Formal analysis AMGL, GRE and LVHC; Funding acquisition: GRE and EEQA; Investigation: AMGL; GAS and CAAG; Methodology: AMGL and CAAG; Supervision: GRE; Validation: GRE, EEQA and LVHC; Writing - original draft: AMGL and GRE; Writing - review and editing: GRE, GAS, EEQA, LVHC.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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